TOXICOLOGIST'S REVIEW

BLA #: 99-1488 (RMS #103949)
SPONSOR: SCHERING-PLOUGH RESEARCH INSTITUTE
PRODUCT: pegylated interferon-α 2b (PEG-IFN, PEG-INTRON™, Schering-Plough)
FORMULATION: lyophilized powder
RELATED DOCUMENTS: [redacted] PLA #83-415 (initial approval of INTRON®-A for hairy cell leukemia); ELA #83-414; PLA #89-0381 (approval of INTRON®-A for hepatitis C)
PROPOSED INDICATION: treatment of chronic hepatitis C infection
ABBREVIATIONS: IFN, recombinant, human interferon-α 2b, derived from *Escherichia coli* (INTRON®-A); PEG, methoxypolyethylene glycol; MIU, 1 x 10^6 anti-viral units of activity (by cytopathic effect bioassay); CHC, chronic hepatitis C virus; PBL, peripheral blood leukocytes; $t½_{elim}$, elimination half-life; AUC, area under the serum concentrations *vs.* time curve; kD, kilodaltons; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NOAEL, no observable adverse effect level; APTT, activated partial thromboplastin time; GGT, γ-glutamyl transpeptidase; GD, gestational day; PBS, phosphate buffered saline, pH 7.0; LDH, lactic dehydrogenase; LD_{50}, dose which causes lethality in 50% of the animals treated; CPE, cytopathic effect; BUN, blood urea nitrogen; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; ELISA, enzyme-linked, immunosorbent assay; RIA, radioimmunoassay

received 12/26/99; assigned 1/4/00; completed 12/19/00

TABLE OF CONTENTS

ABSTRACT:

The safety, biochemical, and pharmacokinetic activities of PEG-IFN and its components SCH 215600 PEG and INTRON®-A were evaluated in ICR mice, Sprague-Dawley rats, New Zealand white rabbits, and cynomolgus and Rhesus monkeys *in vivo*. Because of the species-specificity of IFN, *in vivo* pharmacokinetic, acute, and repeat-dose toxicity testing of PEG-IFN were conducted in rats and cynomolgus monkeys. Pharmacokinetic studies in rats and cynomolgus monkeys demonstrated similar absorption and elimination profiles of PEG-IFN after i/v injection, with an approximate $t\frac{1}{2}_{elim}$ of 25 to 26 h. Systemic exposure in rats after i/v injection, as calculated from the $AUC_{(0-\infty)}$ was increased in a dose-related fashion, was approximately linear, and was similar to that observed in cynomolgus monkeys treated by either i/v or i/m injection. Bioavailability by the s/c route in both rats and cynomolgus monkeys was between 50 and 100%. PEG-INTRON™ has pharmacologic and toxicologic profiles similar to other type I interferons. Major findings in cynomolgus monkeys after repeated, every other day, s/c dosing with PEG-IFN for 4 weeks at 1414, 4239, or 14,130 μg/m^2/dose included decreased food consumption in the absence of significant weight loss, slight to moderate decreases in erythrocyte parameters, platelet and leukocyte counts, and transient elevation in hepatic transaminases (ALT, AST). One female monkey treated with 9 doses of 14.130 μg/m^2/dose PEG-IFN was sacrificed moribund on study d 22, after decreased appetite, scant feces, dehydration, hypothermia, and inactivity were noted on clinical evaluation. Hematologic profiles were unavailable for this animal; however, at week 2 on study, this monkey was found to have 50 to 75% decreases in total leukocyte and absolute neutrophil counts, and slight decreases in erythrocyte parameters. At sacrifice, this animal had several abscesses at the injection sites for PEG-IFN, which on histologic evaluation were associated with moderate to severe, suppurative cellulitis and presence of bacteria in the lesions. No similar changes in appetite, total body weight gain, or hematologic profiles were noted in CD-1(ICR) mice or Sprague-Dawley rats receiving a single i/v or s/c injection of PEG-IFN at doses of up to 60,410 μg/m^2. The decrease in platelets and leukocytes observed in cynomolgus monkeys were related to the dose of PEG-IFN administered, and were only evident during the second and third weeks of treatment. All changes were reversible by the end of the treatment period, with the exception of the red cell losses in several animals, and were correlated with development of anti-interferon neutralizing activity in the serum. Mild to moderate, local irritation and/or inflammation at the site of injection were noted in all groups of PEG-IFN treated monkeys, and in rabbits injected s/c with PEG-IFN in a local irritation study. Histologically, the most consistent finding was evidence of subcutaneous and/or intramuscular hemorrhage at the injection site, without evidence of either acute or chronic inflammation in either rats, rabbits or cynomolgus monkeys treated with single or repeated injections of up to 14,310 μg/m^2/dose PEG-IFN. Sporadic decreases in bone marrow cellularity, lymphoid depletion in the mesenteric nodes and in the spleen, and cortical atrophy in the thymus, as well as evidence of extramedullary hematopoiesis in the liver and spleen were noted in several animals, without an apparent, significant dose-relationship or clinical sequelae. The NOAEL for PEG-IFN in cynomolgus monkeys after repeat, every other day injections for 28 days was 1414 μg/m^2/dose. A loss of detectable IFN activity in the serum and development of neutralizing antibody activity was noted at the end of treatment period in repeat-dose studies in cynomolgus monkeys, with no apparent dose-relationship in either incidence or titer of antibody development induced. PEG-IFN exhibited no evidence of mutagenic potential in five tester strains of *Salmonella typhimurium*, and in *E. coli* strain WP2*uvr*A, using the standard Ames microbial mutagenicity plate incorporation tests. No evidence of clastogenic activity of PEG-IFN was detected in *in vitro* assays using human peripheral blood lymphocytes, or in *in vivo* mouse micronucleus assays. PEG alone had no detectable mutagenic, toxicologic, or teratologic activity

in *in vitro* Ames and human peripheral blood leukocyte assays, an in *in vivo* toxicology testing in CD-1 (ICR) mice, Sprague-Dawley rats, New Zealand white rabbits, and cynomolgus monkeys at doses of up to 1138 $\mu g/m^2$ SCH 215600 s/c, twice weekly for 13 weeks. Treatment of non-pregnant, female cynomolgus monkeys with 52, 262, or 4239 $\mu g/m^2$/dose PEG-IFN every other day for one menstrual cycle inhibited ovarian function in 6/7 monkeys at the highest dose level, as evidenced by lengthening of menstrual cycle duration during the treatment period, irregularities in cycle duration following cessation of treatment, and dose-related decreases in serum estradiol, and progesterone levels. The NOAEL for effects of PEG-IFN on reproductive hormone status and menstrual cycle duration in cynomolgus monkeys was 262 mg/m^2/dose, or approximately 21-fold greater than the recommended human weekly dose of 1 mg/kg.

INTRODUCTION:

The incidence of hepatitis C infection in the United States and worldwide is increasing. In a recent report from the Centers for Disease Control and Prevention, it was estimated that hepatitis C infection was responsible for approximately 150,000 new cases of acute hepatitis each year, in the United States alone. Approximately 1.6% of the population, or 3.5 million patients are estimated to be infected with the virus.

The hepatitis C virus is unique in that it is a single-stranded, RNA-based virus that targets hepatocytes for infection and replication of new virions. About 4 to 8 weeks after the initial HCV infection, acute elevations of hepatic transaminase levels in serum are often noted, signaling that inflammation in the liver is occurring. During this stage of the disease, the majority of patients are asymptomatic, although using sensitive PCR-based assays, HCV RNA may be detected in the serum.

Approximately 80% of patients with acute HCV infection progress to more chronic liver disease. This stage is manifested by persistent elevations in serum levels of hepatic transaminases and HCV RNA. Histologic evidence of chronic inflammatory changes may or may not be present in biopsied liver samples. Further progression of the disease leads to scarring, fibrosis, and cirrhosis in the affected regions of the liver in approximately 20 to 50% of infected patients between 10 to 20 years after the initial infection. At this stage, other clinical features commonly associated with cirrhotic liver disease become evident, such as ascites, jaundice, esophageal varices, and encephalitis. A number of patients with chronic HCV infection may also progress to primary hepatocellular carcinoma.

Treatment options for HCV infection are limited. Consistent decreases in serum transaminase levels, a surrogate marker for hepatic inflammation, have been observed in approximately 50% of patients treated with 3×10^6 IU of recombinant, type I interferons three times weekly for a duration of 6 months. However, between 50 and 75% of the responding patients relapsed after cessation of interferon treatment, resulting in durable response rates of < 25%. Lower doses of interferon, even when administered on a daily schedule were demonstrated to be ineffective when compared to either untreated or placebo-treated control patients. Taken together, these data suggest that continuous, high levels of exposure to type I interferons are necessary for the anti-viral effects in HCV infection.

Pegylated interferon alpha 2b (PEG-INTRON™, PEG-IFN) is a semi-synthetic conjugate of recombinant, *Escherichia coli*-derived interferon-α 2b (INTRON®-A) and polyethylene glycol with an average molecular weight of 12000 daltons. The *in vitro* and *in vivo* properties of PEG-IFN are similar to those of other, recombinant type I interferons. Specifically, PEG-IFN can induce intracellular antiviral activity, inhibit the proliferation of several tumor cell lines, activate natural killer cell-mediated tumor cytolysis, and induce cytokine synthesis and release by immune effector cells similarly to other interferon-α preparations. Its advantages, however, are that it is very slowly cleared after s/c injection, leading to longer terminal half-lives and higher exposure levels (AUC and C_{max}) in both preclinical and clinical pharmacokinetic studies. The resulting increase in exposure is thought to be a major factor in the increased efficacy seen in patients with HCV infection treated with PEG-IFN in the phase 2/3 pivotal trial.

The intended clinical use of PEG-IFN is for the treatment of patients with chronic HCV infection. In the pivotal trial, the safety and efficacy of different doses of PEG-IFN after 48 weeks of treatment and 24 weeks of treatment-free follow-up were compared to those attained in patients treated with the currently licensed, interferon-α 2b regimen (INTRON®-A, Schering). Efficacy in the present study was evaluated by measurement of serum ALT levels over time, as well as quantitation of HCV RNA levels by PCR at baseline, at the end of 24 and 48 weeks of treatment, and at the end of follow-up. The percentages of patients exhibiting normalization of serum ALT levels at the end of the 24 week follow-up period were 24% and 29% for patients treated with 0.5 or 1.0 μg/kg/week PEG-IFN, respectively, as compared to 11% complete responders in the group treated with INTRON®-A. Comparable decreases in serum HCV RNA levels to less than the detectable limits of the assay (considered "HCV RNA-negative") were obtained in both groups of patients (18% and 25 % of patients had undetectable RNA levels when treated with 0.5 or 1.0 μg/kg/week PEG-IFN, as compared to 12% virologic responders in patients treated with 3 MIU INTRON®-A, t.i.w.). Combined response rates for both normalization of ALT and negative HCV RNA responses were 17%, 24%, and 12%, for patients treated with 0.5 or 1.0 μg/kg/week PEG-IFN, or INTRON®-A, respectively.

The dose and schedule of PEG-INTRON™ intended for administration to chronically-infected HCV patients is 1.0 mg/kg, once weekly for 48 weeks. PEG-INTRON™ is formulated with dibasic sodium phosphate, monobasic sodium phosphate, sucrose, and polysorbate 80, and provided as a lyophilized powder for reconstitution with Sterile Water for Injection, U.S.P. The PEG-IFN used for all preclinical pharmacology, pharmacokinetics, and toxicology studies was produced at commercial scale, was greater than 99% pure, was formulated according to clinical procedures and was either of clinical grade, or representative of that used in the clinic.

PRECLINICAL PHARMACOLOGY AND PHARMACOKINETICS:

Pharmacology Study Summary:

1. Ancillary pharmacology of SCH 54031 in rats. Study #P-7011. Charles River CD rats, 6 –7 males/group, weight range 205-350 g; vehicle control (sterile water for injection, U.S.P.), 29,400 μg/m^2 SCH 54031 PEG-IFN (lot #38101-096), s/c; non-GLP; final report dated 9/15/98; Schering-Plough Research Institute, Kenilworth, NJ. **Volume 6, pp. 1-23 (Reference #P-7011).**

2. Ancillary pharmacology of SCH 54031 in monkeys. Study #P-7012. *Macaca fasicularis*, 2-3 adult males/group, weight range 5-7 kg; vehicle control (sterile water for injection, U.S.P.), 14126 μg/m^2 SCH 54031 PEG-IFN (lot #38101-016), s/c; non-GLP; final report dated 9/14/98; Schering-Plough Research Institute, Kenilworth, NJ. **Volume 6, pp. 1-20 (Reference #P-7012).**

3. Antiviral and anti-proliferative activity of PEG_{12000}-interferon-alfa 2b. INTRON®-A, reference standard #00251-1, PEG-IFN, lot #35953-011. **Volume 6, pp. 1-5 (Reference Cullen, C.).**

4. Comparison of MHC Class I expression, NK and LAK activity of Interferon alpha-2b and PEG-Interferon alpha-2b. Study #D-28535. INTRON®-A, lot #2-AVAW-104 (specific activity 2.6 x 10^8 IU/mg protein, 6.54 mg protein/ml); PEG-IFN, lot #6-PPI-101 (specific activity 5.7 x 107 IU/mg, 4.14 mg/ml); non-GLP; Schering-Plough Research Institute, Biotechnology/Bioanalytical Group. **Volume 6, pp. 1-16 (Reference #D-28535).**

Pharmacology Study Review:

Study #P-7011. ***Ancillary pharmacology of SCH 54031 in rats.***

The effects of treatment with PEG-IFN on blood pressure, electrocardiographic profiles, gastric motility, renal function, and neurobehavioral function were evaluated in male Sprague-Dawley rats after either a single, s/c administration.

Cardiovascular parameters were measured in conscious, normotensive rats after an overnight fast. Rats had previously been cannulated in the caudal artery for measurement of arterial blood pressure using a Spectromed pressure transducer connected to a computer-based analyzer. Electrocardiographic leads were implanted on the right and left forelegs and the left rear leg, and hemodynamic parameters were monitored at baseline (immediately prior to dosing). Following s/c injection with 29,400 μg/m^2 PEG-IFN, heart rate, blood pressure, and electrocardiograms were determined at 30 min intervals for up to 12 h post-dosing.

There were no treatment-related effects of PEG-IFN on blood pressure, as compared to animals treated with the vehicle control. A trend towards decreased heart rate was observed in the PEG-IFN treated rats. However, this finding was statistically significantly different from control at only the 3.5 hour time point (mean decrease from baseline of -29 ± 9 bpm in the PEG-IFN treated rats, as compared to $+14 \pm 15$ bpm in the vehicle control group). There were no significant changes in the electrocardiographic profiles, nor the PR, QRS, QT, or RR intervals in either the vehicle control or the PEG-IFN treated rats. There were no signs of AV nodal block, absent P waves, or blunting of the QRS complex in PEG-IFN treated rats, as compared to the vehicle control group. Taken together, these data suggest that the apparent bradycardia observed in the PEG-IFN treated rats, which was not associated with a prolonged RR interval is of minimal toxicologic significance.

Gastric motility was determined in male rats after s/c injection of the vehicle control, 29,400 μg/m^2 PEG-IFN, or 1 mg/kg atropine as a positive control. Six rats per group were injected with the control or test article, and 3 ml of a standardized, test meal was administered by gavage 12 hours after injection. Exactly 30 min later, rats were killed by cervical dislocation, the full stomachs were ligated at the pylorus and esophagus, excised, and weighed. The stomachs were then cut

open, rinsed, and weighed again. Gastric emptying was calculated by subtracting the difference in weights between the full and empty stomachs from the weight of the 3 ml test meal, divided by the weight of the 3 ml test meal, and reported as the percent emptied.

Treatment of rats with PEG-IFN did not affect gastric motility as compared to rats treated with the vehicle control. By contrast, gastric emptying was inhibited by approximately 49% in rats treated with 1 mg/kg atropine 12 h prior to the test meal. The NOAEL for SCH 54031 PEG-IFN on gastric motility is therefore 29,400 $\mu g/m^2$.

Six rats per group were treated with vehicle control, 29,400 $\mu g/m^2$ PEG-IFN, or 3 mg/kg furosemide as a positive control, for the evaluation of renal function. All rats were supplemented with 15 ml/kg physiologic saline, p/o prior to dosing with the control or test articles. Following dosing, rats were placed in metabolic cages and urine was collected continuously for 6 hours after treatment, and then from 7 to 24 hours. Total urine volume, sodium and potassium concentration, and creatinine levels were determined for each time point. Additionally, serum creatinine was measure at 24 h, and the endogenous creatinine clearance as a measure of glomerular filtration rate was calculated.

There were no differences between the vehicle control and the PEG-IFN-treated rats in total urine volume, excretion of sodium and potassium, and creatinine clearance at both the 6 and 24 hour time points. Urinalysis profiles were negative for glucose, bilirubin, and blood and there were no differences between the groups in urinary pH and ketones. The NOAEL for PEG-IFN effects on renal function in rats, under the conditions of this assay is therefore 29,400 $\mu g/m^2$, as a single s/c injection.

Behavioral, neurologic, and autonomic function were evaluated at 1, 2, 4, 6, 8, 10, and 12 h after dosing, using a modification of the method of Irwin[1]. Following dosing with either the vehicle control or PEG-IFN, the rats were observed for signs of neurologic toxicity (*e.g.* convulsions, tremors), and for normal neurologic and behavioral parameters (*e.g.* spontaneous activity, alertness, and pupil size). Each sign was assigned a quantitative score of 0, or ± 1, ± 2, or ± 3, indicating slight, moderate, or marked increases or decreases from normality.

There were no remarkable effects of treatment with PEG-IFN on behavioral, neurologic, and autonomic function at any time point after injection, as compared to rats treated with the vehicle control. No lethality or clinical signs of toxicity were noted over the 24 h observation period.

In summary, treatment of rats with 29,400 $\mu g/m^2$ PEG-IFN was not associated with any cardiovascular, hemodynamic, neurologic or behavioral toxicities, or effects on gastric motility.

Comment: Previous preclinical *in vitro* and *in vivo* studies with the unconjugated, human interferon in rats and mice have demonstrated that the biologic is inactive in rodent species. Therefore, the safety pharmacology studies conducted in rats are irrelevant, and do not provide any useful information in determining the effects of PEG-IFN *in vivo*.

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[1] Irwin, S. 1964. Drug screening and evaluation of new drugs in animals. *In*: Animal and Clinical Pharmacologic Techniques in Drug Evaluation, J.M. Nodine and P.I. Siegler, eds., Year Book Medical Publishers, Inc., Chicago, IL; pp. 36-64.

Study #P-7012. ***Ancillary pharmacology of SCH 54031 in monkeys.***

The effects of treatment with PEG-IFN on blood pressure, electrocardiographic profiles, and neurobehavioral function were evaluated in male cynomolgus monkeys after either a single, s/c administration.

Cardiovascular parameters were measured in conscious, free-roaming monkeys for one hour prior to dosing and for 6 hours following administration of PEG-IFN. Monkeys had been previously surgically implanted with radiotelemetric cardiovascular transmitters and electrocardiogram leads for measurement of hemodynamic parameters and ECG profiles. Following s/c injection with 14,126 $\mu g/m^2$ PEG-IFN or an equivalent volume of vehicle control (sterile water for injection, U.S.P.), heart rate, blood pressure, and electrocardiograms were determined at 10 min intervals for up to 6 h post-dosing. After a one week washout period, animals were crossed over to the opposite treatment group, and administration of the test or control articles was repeated.

Because of a high signal-to-noise ratio obtained in the conscious, free-roaming monkeys, ECG profiles (*i.e.* P and T wave profiles) could not be reliably determined from the above measurements. Electrocardiograms were repeated as a separate study in conscious, chair-restrained monkeys several weeks after completion of the initial study. Six-lead ECG profiles were obtained using ECG limb leads rather than the implanted electrodes, and were recorded on a Burdick Eclipse-850 clinical monitor. Measurements for this study were determined only at baseline (immediately prior to dosing) and at 6 hours later. Monkeys were again crossed over to the opposite treatment groups and the measurements repeated one week later.

There were no treatment-related effects of PEG-IFN on mean blood pressure over the 6 h monitoring period, as compared to animals treated with the vehicle control. Significantly higher heart rates were observed in the monkeys treated with PEG-IFN at 2 hours after injection, and lasting through the remainder of the monitoring periods. Heart rates in the PEG-IFN treated animals were elevated by approximately 40-90 bpm, as compared to the monkeys treated with the vehicle control at the same time points ($p \leq 0.05$, ANOVA and Scheffee's statistic). Body temperatures were also increased at these time points in the PEG-IFN treated monkeys, as compared to either baseline or to the vehicle control monkeys. There were no significant changes in the electrocardiographic profiles, nor the PR, QRS, QT, or RR intervals in either the vehicle control or the PEG-IFN treated monkeys. Chair-restrained monkeys had a basal heart rate of approximately 220 bpm, as compared to the basal rate of 115-130 bpm in the free-roaming animals. The RR interval at 6 h was not increased in the chaired monkeys at the 6 h time point, as compared to the elevated heart rates reported in the free-roaming animals over the duration of the measurements. There were no signs of AV nodal block, absent P waves, or blunting of the QRS complex in the chair-restrained, PEG-IFN treated monkeys, as compared to the vehicle control group. Taken together, these data suggest that the apparent tachycardia observed in the PEG-IFN treated monkeys, which was not associated with a decreased RR interval is of minimal toxicologic significance.

Behavioral, neurologic, and autonomic function were evaluated at 30, 60, 120, 240, and 360 minutes, and 24 h after dosing, using a modification of the method of Irwin[1]. Following dosing with either the vehicle control or PEG-IFN, the monkeys were observed for a panel of 24 distinct behavioral, neurologic, and autonomic functions as signs of neurologic toxicity (*e.g.* spontaneous activity, alertness, and pupil size). A quantitative score of 0 to 5 was given for each parameter at every time point, with a score of ≥ 2 counted as one occurrence.

There were no remarkable effects of treatment with PEG-IFN on behavioral, neurologic, and autonomic function at any time point after injection, as compared to monkeys treated with the vehicle control. No lethality or clinical signs of toxicity were noted over the 24 h observation period; however, one of the three monkeys treated with PEG-IFN developed diarrhea at 24 h after injection.

In summary, treatment of conscious, adult male cynomolgus monkeys with 14,126 $\mu g/m^2$ PEG-IFN was not associated with any cardiovascular, hemodynamic, neurologic or behavioral toxicities, under the conditions of the assays employed here.

Cullen, C. ***Antiviral and antiproliferative in vitro biologic activity of PEG_{12000}-Interferon-alfa 2b.***

The anti-viral and anti-proliferative activities of PEG-IFN as compared to unconjugated, INTRON®-A IFN were determined in a series of *in vitro* assays. For the anti-viral assay, confluent monolayers of cultured, human foreskin fibroblasts were treated with serial, 1:2 dilutions of either INTRON®-A or PEG-IFN for several hours, prior to infection with encephalomyocarditis virus (EMCV). Incubation in the presence of both IFN and ECMV was continued for 18-24 h, the cultures were fixed in methanol, stained with MTT, and the optical density at 570 nm (OD_{570}) determined. Cell viability, as determined by MTT dye uptake, was directly proportional to the degree of viral inhibition by the IFN preparations, and was plotted against a standard curve.

Titration of PEG-IFN against the INTRON®-A standard demonstrated that the two preparations have virtually identical antiviral activity. Curves for OD_{570} against dilution were superimposable for the two IFN moieties.

Comment: The starting concentrations of PEG-IFN and INTRON®-A used in the anti-viral assay were not specified in the final report. The stock concentrations were 4.3 mg/ml for the PEG-IFN lot #35953-011 used in the assay, and 35.01 ng/vial INTRON®-A for the reference standard.

Inhibition of growth the human B-cell lymphoma Daudi cell line in the presence of PEG-IFN was compared to the anti-proliferative effects of unconjugated, INTRON®-A. Serial, 1:2 dilutions of either INTRON®-A or PEG-IFN were added to Daudi cells in culture medium, and the plates were incubated for 72 hours. At the end of the incubation period, the cultures were fixed and stained with MTT, and the optical density at 570 nm (OD_{570}) determined. Inhibition of Daudi cell proliferation, as determined by decreased MTT dye uptake, was directly proportional to the amount of IFN in the preparations, and was plotted against a standard curve.

Titration of PEG-IFN against the INTRON®-A standard demonstrated that the two preparations have virtually identical anti-proliferative activity in human Daudi B-cell lymphoma cell lines. Curves for OD_{570} against dilution were superimposable for the two IFN moieties.

Comment: The starting concentrations of PEG-IFN and INTRON®-A used in the anti-proliferative assay were not specified in the final report. The stock concentrations were 4.3 mg/ml for the PEG-IFN lot #35953-011 used in the assay, and 35.01 ng/vial INTRON®-A for the reference standard.

In summary, the anti-viral and anti-proliferative activities of PEG-IFN were virtually identical to those of the reference standard, INTRON®-A, confirming the biologic activity of the PEG-modified protein.

Study #D-28535. ***Comparison of MHC Class I expression, NK and LAK activity of interferon alpha-2b and PEG-interferon alpha-2b.***

The ability of PEG-IFN to induce several of the pharmacodynamic markers of IFN activity was compared to the effects obtained with INTRON®-A in three, *in vitro* cellular assays. Major histocompatibility (MCH) Class I antigen expression was determined using the human MOLT-4 T cell line, followed by flow cytometric evaluation of cells stained for expression of human HLA-A, HLA-B, and HLA-C. Cytolytic activity of natural killer (NK) cells and lymphokine-activated killer (LAK) cells was determined in a non-radioactive, *in vitro* CytoTox-96® LDH-release assay, with the human K562 chronic myelogenous leukemia cells as targets for NK lytic activity, and the Daudi B-cell (Burkitt's) lymphoma cells used as targets for LAK cell killing. Human peripheral blood lymphocytes were used without further purification for the NK assay, and after culture for 72 h in the presence of 4 or 200 U/ml human interleukin-2, with or without added IFN or PEG-IFN.

Both IFN and PEG-IFN induced the expression of MHC Class I antigens on the surface of MOLT-4 T cells in a dose-dependent manner, as detected by flow cytometry. The EC_{50} for MHC Class I expression by PEG-IFN was 0.74 ng/ml, as compared to a value of approximately 0.16 ng/ml for unmodified INTRON®-A. When concentrations of IFN and PEG-IFN were adjusted for anti-viral activity and the same levels added to MOLT-4 cells, comparable expression of MHC Class I activity was induced by the same doses of either formulation.

No significant differences were noted in the ability of PEG-IFN or INTRON®-A to induce NK cell or LAK cell cytotoxicity. Both agents were capable of stimulating lytic activity by 3 to 14-fold over baseline after a 16 to 72 h incubation with the donor cells. However, a high degree of donor-to-donor variability was observed, therefore, no definitive conclusions about the comparability of the two agents could be made.

In summary, PEG-IFN was found to induce several pharmacodynamic markers of interferon activity, including MHC Class I expression, NK cytolysis and LAK cell lytic activity similarly to treatment with INTRON®-A.

Pharmacokinetics Study Summary:

1. SCH 54031: Pharmacokinetics of PEG_{12000}-IFN-α 2b administered by subcutaneous or intravenous injection to male rats. Study #SN 95311 (Report #P-6125). Crl:CD®(SD)BR VAF/Plus™ rats (weight range 231-330 g), 3 ♂/dose/time point; 2.91 x 10^8, 8.16, 16.8 x 10^8 IU/m^2 (4914, 14280, 29400 μg/m^2) PEG_{12000}-IFN-α 2b, lot #33208-110-03, s/c; 2.81 x 10^8 IU/m^2 (4914 μg/m^2) PEG_{12000}-IFN-α 2b, lot #33208-110-03, i/v; or 2.77 x 10^8 IU/m^2 (1386 μg/m^2) INTRON®-A, lot #4-IFF-001, i/v or s/c; non-GLP; 3/8-10/31/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 25, pp. 1-51 (Reference 1).**

2. SCH54031: Pharmacokinetics of PEG_{12000}-IFN-α 2b administered by subcutaneous injection to male cynomolgus monkeys. Study #SN 94454 (report #P-6109). *Macaca fasicularis* (weight range 3.2-5.0 kg), 4 ♂/group; 1413, 4238, 14126 μg/m^2 PEG_{12000}-IFN-α 2b, lot #33208-110-03, s/c; 1413 μg/m^2 PEG_{12000}-IFN-α 2b, lot #33208-110-03, i/v; 1345 μg/m^2 INTRON®-A, lot #4-IFF-001, s/c; non-GLP; 2/6-2/13/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 25, pp. 1-94 (Reference 2)**.

3. SCH54031: Evaluation of batch to batch reproducibility of PEG_{12000}-IFN-α 2b pharmacokinetics in cynomolgus monkeys following a single subcutaneous administration. Study #SN 95307 (Report #P-6191). *Macaca fasicularis* (weight range 3.4-4.8 kg), 6 ♂/group; 4237 μg/m^2 PEG_{12000}-IFN-α 2b, lot #50569-053-1B; 4237 μg/m^2 PEG_{12000}-IFN-α 2b, lot #50569-053-1A; non-GLP; 5/8-11/6/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 25, pp. 1-144 (Reference 3)**.

4. SCH54031: Subcutaneous single dose tolerance study of SCH 54031 (PEG_{12000}-IFN-α 2b) in cynomolgus monkeys. Study #P-6135. *Macaca fasicularis* (weight range 2.8-3.6 kg ♂, 2.7-3.4 kg, ♀), 2/sex/group; 29435 (♀ only), 58861, 117721 μg/m^2 SCH54031, lot #33208-157; GLP; 4/13-11/17/95; Schering-Plough Research Institute, Lafayette, NJ. (**Please note** - is same study as toxicology study #5, Schering Study #95028 [Report #P-6135]). **Volume 7, pp. 1-185 (Reference 5)**.

5. SCH54031: One month subcutaneous toxicokinetic study of SCH 54031 (PEG_{12000}-IFN-α 2b) solution in cynomolgus monkeys. Study #P-6151. *Macaca fasicularis* (weight range 2.3-3.7 kg, ♂, 2.2-3.7 kg, ♀), 3/sex/group; vehicle control (IFN placebo, lot #35293-001), 1414, 4239, 14130 μg/m^2 SCH54031, lot #33208-157, or 3105 μg/m^2 INTRON®-A, lot #33208-155-03; GLP; 4/4-11/7/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 8, pp. 1-308 and Volume 9, pp. 309-527 (Reference 6)**. (**Please note** - is same study as toxicology study #6, Schering Study #94081 [Report #P-6136]).

6. SCH54031: Tissue distribution of radioactivity by whole body autoradiography following a single administration of ^{125}I-interferon-α 2b or ^{125}I-PEG_{12000}-IFN-α 2b. Study #D-27156. Sprague-Dawley rat, 2 ♂/dose/time point; 1386 μg/m^2 ^{125}I-INTRON®-A (approximately 50 μCi), lot #, 4914 μg/m^2 ^{125}I-SCH54031, lot #; 50 μCi ^{125}I-sodium periodide; 50 μCi ^{125}I-N-succinimidyl-*p*-iodobenzoate; sacrifice 1, 4, 24, 72, 168 h after injection (72, 168 h time points for PEG-IFN only); non-GLP; Schering-Plough Research Institute, Lafayette, NJ. **Volume 25, pp. 1-45 (Reference 6)**.

7. SCH 54031: Metabolism and excretion of PEG_{12000}[^{125}I]-IFN α-2b following a single subcutaneous dose to male cynomolgus monkeys. Study #98183 [redacted]; Document [redacted] *Macaca fasicularis*, 3 ♂/group; 1413 μg/m^2 ^{125}I-labeled SCH 54031 PEG-IFN, s/c (lot #7-PPI-105; specific activity 608 μCi/mg protein); GLP; 8/24/98 – 3/1/99; [redacted] **Volume 25, pp. 1-119 (Reference 7)**.

Pharmacokinetics Study Review:

Study #SN 95311 (Report #P-6125). ***SCH 54031: Pharmacokinetics of PEG_{12000}-IFN-α 2b administered by subcutaneous or intravenous injection to male rats.***

The pharmacokinetics of SCH 54031 PEG-IFN were initially evaluated in male, Sprague-Dawley rats after a single s/c or i/v injection. Fifteen rats per group were treated with 281, 816, or 1680 x 10^6 IU/m^2 (4914, 14280, or 29400 μg/m^2, respectively) PEG-IFN by s/c injection, or 281 x 10^6 IU/m^2 PEG-IFN, i/v. An additional 15 rats per group were treated with 277 MIU/m^2 unconjugated INTRON®-A as a comparative control. Three rats per dose group were sacrificed by CO_2 inhalation and blood collected by cardiac puncture at 1, 2, 3, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h after injection of PEG-IFN or INTRON®-A. Urine samples were also collected over the course of the study at 24 h intervals, from the group of rats sacrificed at 168 h after treatment; however, PEG IFN was subsequently determined to be unstable in rat urine, so the samples were not analyzed.

Serum samples were analyzed for interferon activity by both ELISA and by inhibition of the cytopathic effects of encephalomyocarditis virus (ECMV) on cultured, FS-4 human foreskin fibroblasts (CPE bioassay). In this assay system, one unit of interferon activity is defined as the amount to inhibit the cytopathic effect by 50%, as compared to cultures of FS-4 cells exposed to ECMV in the absence of IFN. Pharmacokinetic parameters were calculated from the serum IFN concentration *vs.* time profiles for all groups, using nonparametric, model-independent analyses.

After a single, i/v administration of PEG-IFN, interferon activity could be detected in the rats up to 168 hours after dosing. Peak concentrations were observed at the first sampling time point of 0.25 h, then declined in a biphasic manner. Terminal elimination half-life after i/v injection was determined to be approximately 25 h using data from the ELISA assay, and 13 h when the CPE data were used to calculate the pharmacokinetic profiles.

A different pattern of exposure was observed following s/c injection of PEG-IFN. In general, the calculated pharmacokinetic parameters for PEG-IFN after s/c injection were similar when analyzed by either the ELISA or CPE bioassay. Serum IFN levels increased slowly over time; T_{max} was not observed until 12 h after dosing in all groups of rats treated with PEG-IFN, with terminal half-lives ranging from 18 to 24 h (by ELISA). Peak concentrations, however, were approximately 15 to 20-fold lower after s/c injection than following i/v injection of the same dose of PEG-IFN. Dose-related increases in both C_{max} and AUC (either to final time or extrapolated to infinity) were observed for all doses of PEG-IFN, and were approximately linearly related to the dose of PEG-IFN injected. Bioavailability of PEG-IFN, as determined by the ratios of AUC after s/c and i/v injections, was approximately 51% by ELISA and 43% by CPE assay. The data for the ELISA assay only are presented in Table I, below:

Table I – Pharmacokinetic Profile of PEG-IFN in Sprague-Dawley Rats

P/K Parameter	Mean Value for Pharmacokinetic Parameters (n = 3/time point)					
	PEG-IFN Dose Level				INTRON®-A Dose Level	
	4914 μg/m², i/v	4914 μg/m², s/c	14280 μg/m², s/c	29400 μg/m², s/c	1386 μg/m², i/v	1386 μg/m², s/c
C_{max} (IU/ml)	649876	37861	95943	175710	82368	14446
T_{max} (h)	0.25	12	12	12	0.25	1
$t1/2_{elim}$ (h)	24.64	20.69	23.51	17.68	8.33	0.97
$AUC_{(0\text{-}tf)}$ (IU·hr/ml)	2111899	1061761	3055950	6369048	52715	58649
$AUC_{(0\text{-}\infty)}$ (IU·hr/ml)	2118859	1064825	3068935	6379792	53135	58675
F_{abs} (%)[a]	n.a.[b]	50.3	49.8	50.3	n.a.[b]	110

[a] bioavailability calculated from ratio of $AUC_{(0\text{-}\infty)}$ by s/c and i/v injection and corrected for dose
[b] n.a. = not applicable to calculate this parameter for i/v injection

By contrast, both lower serum levels and total exposure were obtained in rats after either i/v or s/c injection of INTRON®-A. Peak serum concentrations were achieved by 0.25 h after i/v injection and at 1 h after s/c injection. The final times for detectable IFN activity were 24 and 12 h, respectively. Total exposure to IFN, as determined by the calculated AUCs was approximately equal for both routes of administration, with a bioavailability of greater than 100%.

In summary, treatment of male Sprague-Dawley rats with PEG-IFN resulted in a more prolonged exposure to IFN activity than that observed following injection of INTRON®-A. Dose-related increases in both serum IFN concentrations and total exposure, as determined by AUC were observed for rats treated with PEG-IFN, and the material was approximately 50% bioavailable after s/c injection, as compared to after i/v administration.

Study #SN 94454 (report #P-6109). ***SCH54031: Pharmacokinetics of PEG_{12000}-IFN-α 2b administered by subcutaneous injection to male cynomolgus monkeys.***

The absolute bioavailability, pharmacokinetics, and dose proportionality of PEG-IFN was determined in male cynomolgus monkeys, following a single i/v or s/c administration. Four monkeys per group were treated with 1413, 4238, or 14126 μg/m², PEG-IFN by s/c injection, or 1413 μg/m² PEG-IFN, i/v. An additional four monkeys were treated with 1345 μg/m² unconjugated INTRON®-A, as a comparative control. Peripheral blood samples were obtained from each monkey at 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, 144, and 168 h after injection of PEG-IFN or INTRON®-A. Additional samples at 0.25 and 0.5 h were obtained from the animals treated with PEG-IFN by i/v injection. Urine samples were also collected over the course of the study immediately prior to dosing, and in intervals from 0-4, 4-8, and 8-24 h after injection of PEG-IFN, then at 24 h intervals until termination of the study. Urine volumes were recorded, and 25 ml aliquots stored at –70°C for further analysis. At the completion of the study, all monkeys were returned to the research colony.

Both urine and serum samples from PEG-IFN treated monkeys were analyzed for interferon activity by ELISA and by inhibition of the cytopathic effects of ECMV on cultured, human foreskin fibroblasts (CPE bioassay). In this assay system, one unit of interferon activity is defined as the amount required to inhibit the cytopathic effect by 50%, as compared to cells exposed to ECMV in the absence of IFN. Pharmacokinetic parameters were calculated from the serum IFN concentration *vs.* time profiles for all groups, using nonparametric, model-independent analyses.

All monkeys survived the dosing and observation periods with no apparent clinical signs of toxicity. After a single, i/v administration of PEG-IFN, interferon activity could be detected in the monkeys up to 120 hours after dosing. Peak concentrations were observed at the first sampling time point of 0.25 h, then declined gradually over the duration of the study. Terminal elimination half-life after i/v injection was determined to be approximately 26 h using data from the ELISA assay, and 14 h when the CPE data were used to calculate the pharmacokinetic profiles.

Following s/c injection of PEG-IFN, the calculated pharmacokinetic parameters for PEG-IFN after s/c injection were slightly increased when analyzed by the ELISA assay, as compared to values obtained when the CPE bioassay was used for analysis. Serum IFN levels increased slowly over time, with T_{max} observed at 4-6 h after dosing in all groups of monkeys treated with PEG-IFN, and terminal half-lives ranging from 29 to 34 h (by ELISA). Peak concentrations, however, were approximately 15 to 17-fold lower after s/c injection than following i/v injection of the same dose of PEG-IFN. Dose-related increases in both C_{max} and AUC (either to final time or extrapolated to infinity) were slightly greater than linearly related to the dose of PEG-IFN injected. Bioavailability of PEG-IFN, as determined by the ratios of AUC after s/c and i/v injections, was increased in proportion to the dose of PEG-IFN administered, and ranged from 57 to 89% by ELISA and 55 to 97% by CPE assay. The data for the ELISA assay only are presented in Table II, below:

Table II – Pharmacokinetic Profile of PEG-IFN in Male Cynomolgus Monkeys

P/K Parameter	Mean Value for Pharmacokinetic Parameters, ± Percent C/V				
	PEG-IFN Dose Level				INTRON®-A
	1413 µg/m², i/v	1413 µg/m², s/c	4238 µg/m², s/c	14126 µg/m², s/c	1345 µg/m², s/c
C_{max} (IU/ml)	175400 ± 8	11410 ± 22	49239 ± 43	157539 ± 54	20844 ± 39
T_{max} (h)	0.5 ± 0	4.5 ± 43	5.25 ± 29	5.5 ± 87	2.8 ± 18
$t1/2_{elim}$ (h)	26.3 ± 10	29.5 ± 14	29.2 ± 8	34.1 ± 9	3.6 ± 14
$AUC_{(0\text{-}tf)}$ (IU·hr/ml)	688452 ± 13	386768 ± 19	1586996 ± 23	5923529 ± 21	183048 ± 36
$AUC_{(0\text{-}\infty)}$ (IU·hr/ml)	693411 ± 13	396306 ± 19	1623720 ± 21	6179938 ± 21	18530 ± 36
F_{abs} (%)[a]	n.a.[a]	57.2[b]	78.1	89.1	n.d.[c]

[a] n.a. = not applicable to calculate this parameter for i/v injection

[b] bioavailability calculated from ratio of mean $AUC_{(0\text{-}\infty)}$ by s/c and i/v injection and corrected for dose

[c] n.d. = not done

Peak serum concentrations after s/c injection of INTRON®-A were achieved by 2 h, and the final time for detectable IFN activity was 24 h after dosing. Total exposure after injection of 1345 µg/m² IFN, s/c, as determined by the calculated AUC, was approximately 50% of that achieved

after dosing with a similar dose of PEG-IFN (1413 μg/m^2, s/c). In contrast to the relatively long elimination half-life of PEG-IFN, the terminal elimination half-life of INTRON®-A in cynomolgus monkeys was 3.6 hours. These values are similar to those previously obtained for IFN in Rhesus or cynomolgus monkeys (data not shown).

In summary, treatment of male, cynomolgus monkeys with PEG-IFN resulted in both an increased, as well as a more prolonged exposure to IFN activity than that observed following injection of INTRON®-A. Dose-related increases in both serum IFN concentrations and total exposure, as determined by AUC were observed for monkeys treated with PEG-IFN, and the material was approximately 50% to 97% bioavailable after s/c injection, as compared to after i/v administration.

Comment: Both the protocol and the final study report state that urine samples were collected and archived for analysis of urinary IFN activity. However, there are no data in the final report that indicate that these samples were ever analyzed, and no amendments to the protocol are included which would provide an explanation for why these assays were not done.

Study #SN 95307 (Report #P-6191). ***SCH54031: Evaluation of batch to batch reproducibility of PEG$_{12000}$-IFN-α 2b pharmacokinetics in cynomolgus monkeys following a single subcutaneous administration.***

The comparability of two different lots of PEG-IFN was determined after s/c injection and exposure in male cynomolgus monkeys. Six animals per group were dosed with a single injection of 4237 μg/m^2 PEG-IFN, from lots #50569-053-1B, or #50569-053-1A. Peripheral blood samples for determination of IFN serum levels were collected at baseline, then immediately prior to dosing with PEG-IFN, and at selected time points up to 336 hours after treatment. Serum samples from the different time points were analyzed for IFN activity by both CPE bioassay and ELISA. Samples of peripheral blood for determination of anti-IFN neutralizing antibody activity were also obtained at various time points on study, and analyzed for their ability to inhibit the protective effects of IFN in the CPE bioassay.

Comparability of the two lots of PEG-IFN was determined by comparing the C_{max} and $AUC_{(0-\infty)}$ generated for each of the groups of treated monkeys. Data generated by the ELISA and the CPE bioassays are presented in Tables IIIA and IIIB, respectively, below:

Table III – Pharmacokinetic Comparability of Two Lots of PEG-IFN in Monkeys

A. ELISA Assay for IFN levels

P/K Parameter	PEG-IFN Preparation Tested	
	Lot #50569-053-1B	Lot #50569-053-1A
	Mean Value ± % C.V.	Mean Value ± % C.V.
C_{max} (IU/ml)	29159 ± 34	19069 ± 26
T_{max} (h)	4.8 ± 56	8.7 ± 90[a]
$t1/2_{elim}$ (h)	26.2 ± 16	32.5 ± 21
$AUC_{(0-tf)}$ (IU·hr/ml)	786570 ± 16	779850 ± 12
$AUC_{(0-\infty)}$ (IU·hr/ml)	796100 ± 16	798490 ± 12

[a] T_{max} = 5.6 h ± 34% C.V. if exclude animal #11M (T_{max} was 24 h by ELISA)

B. CPE Assay for IFN levels

P/K Parameter	PEG-IFN Preparation Tested	
	Lot #50569-053-1B	Lot #50569-053-1A
	Mean Value ± % C.V.	Mean Value ± % C.V.
C_{max} (IU/ml)	44800 ± 35	36800 ± 35
T_{max} (h)	2.8 ± 41	3.7 ± 66
$t1/2_{elim}$ (h)	16.1 ± 14	15.9 ± 11
$AUC_{(0\text{-}tf)}$ (IU·hr/ml)	1079700 ± 5	1153200 ± 7
$AUC_{(0\text{-}\infty)}$ (IU·hr/ml)	1081800 ± 5	1154300 ± 7

Following s/c injection of lot #50569-053-1A, an 18% and a 35% decrease in the mean values for C_{max}, as detected by the CPE bioassay and ELISA, respectively, was observed as compared to the values obtained for the same dose of lot #50569-053-1B. This finding may be attributed to somewhat slower absorption of lot #50569-053-1A after s/c injection, as evidenced by a 30 to 81% increase in the time to maximal serum concentration (T_{max}) of PEG-IFN in this group, compared to the group of animals treated with lot #50569-053-1B. However, total exposure levels, as determined by $AUC_{(0\text{-}\infty)}$ were within the acceptable range from demonstration of bioequivalence, differing by less than 10% for the two PEG-IFN preparations.

In summary, s/c injection of two different preparations of PEG-IFN in male, cynomolgus monkeys resulted in comparable exposure levels, as determined by the ratio of $AUC_{(0\text{-}\infty)}$, although there were differences observed in both the time to peak serum concentration of IFN, as well as the actual C_{max} values achieved.

Comment: Based on the data presented for C_{max} values, this reviewer would not consider these two preparations to be comparable. It is generally accepted that the toxicities of the type I interferons are related to the C_{max} values, and not to AUC. Therefore, lot #50569-053-1B would be expected to demonstrate greater toxicity in *in vivo* assays that would lot #50569-053-1A. However, review of the toxicology studies included in the submission (below) shows that these two lots were not tested for their ability to induce toxicity in cynomolgus monkeys.

Comment: Both the protocol and the final study report state that peripheral blood samples were obtained for analysis of IFN neutralizing antibody activity. However, the results of these assays were not provided in the final, audited study report.

Study #P-6135. ***SCH54031: Subcutaneous single dose tolerance study of SCH 54031 (PEG_{12000}-IFN-α 2b) in cynomolgus monkeys.***

The toxicokinetic profile of SCH 54031 PEG-IFN was evaluated in male and female cynomolgus monkeys as part of a single, s/c acute toxicity study (please see Study #95028 [Report #P-6135], below). Two animals per sex received a single injection of 29435, 58861, or 117,721 μg/m^2 PEG-IFN subcutaneously into the interscapular region. Samples of peripheral blood for determination of serum IFN and anti-interferon neutralizing antibody activity were obtained from all monkeys on study, with the exception of one female monkey (animal #3F), which was sacrificed moribund on

day 7. Serum interferon levels were determined by both ELISA and CPE bioassay from samples collected prior to dosing on day 1, and at 6 and 24 hours, and 7, 14, and 21 days post-dose. Samples for evaluation of anti-interferon neutralizing activity were collected prior to dosing on day 1, and at study termination on day 21, and evaluated by both enzyme immunoassay and CPE bioassay for neutralization of the anti-viral activity of IFN.

Systemic exposure to IFN was found to be dose-related, following a single, s/c injection of SCH 54031 PEG-IFN. Both C_{max} and AUC were increased in a dose-related manner, as detected by both the ELISA and CPE bioassay. Serum levels of interferon activity were quantifiable on days 1 and 7 of study in all monkeys, but were no longer detectable within the limits of either assay by day 14. The loss of detectable interferon activity may have been due to the presence of neutralizing antibody activity, which can impede the detection of interferon bioactivity. The values obtained for day 1 are presented in Table IV, below:

Table IV - Toxicokinetic Evaluation of PEG-IFN in Cynomolgus Monkeys by ELISA

T/K Parameter	Mean Value, ± C.V. (%)		
	29440 µg/m²	**58860 µg/m²**	**117700 µg/m²**
C_{max} (IU/ml)	148712 ± 27	249121 ± 22	542207 ± 26
T_{max} (hours)	6 ± 0	6 ± 0	11 ± 86[a]
$AUC_{(0\text{-}24hr)}$ (IU·hr/ml)	2505680 ± 27	4560000 ± 16	10069000 ± 24

[a] T_{max} 6 hours in 3/ 4 monkeys, 24 hours in 1/ 4 monkeys

By day 7, mean serum interferon concentrations, as determined by ELISA had decreased to 2536, 14,549, and 25,292 IU/ml in all surviving animals in the low, mid- and high-dose groups, respectively. Similar results were obtained when the CPE bioassay was used to quantitate the serum levels of interferon activity; however, in the mid- and high-dose samples, the serum concentrations by CPE bioassay were approximately 2-fold higher than those obtained by ELISA (data not shown).

Comment: The discrepancies between the two assays were felt by the sponsor to be related to the fact that SCH 54031 has approximately 20% of the activity of free IFN in the CPE bioassay, while the ELISA will detect both free IFN and PEG-IFN at the same intensity.

Neutralizing activity to interferon was detected in all surviving animals at all dose levels of PEG-IFN at day 21 on study, with the exception of one male and one female monkey in the mid-dose group. Quantitation of neutralizing titer by ELISA was not performed. Titers by the CPE bioassay ranged between 1:5 to 1:80, and were unrelated to the dose of PEG-IFN administered.

Comment: Serum neutralizing activity was not determined for the two male monkeys treated with 29,440 µg/m² SCH 54031 PEG-IFN. This was not recorded as a deviation from protocol in the final, audited study report.

Study #P-6151. ***SCH54031: One month subcutaneous toxicokinetic study of SCH 54031 (PEG_{12000}-IFN-α 2b) solution in cynomolgus monkeys.***

The toxicokinetic profile of PEG-IFN was evaluated as part of a one month, repeat-dose toxicity study in cynomolgus monkeys (please see Study #94081 [Report #P-6136], below). Three monkeys/sex were treated with s/c injections of vehicle control (SCH 54031 placebo), 1414, 4239, or 14,130 μg/m^2 PEG-IFN. An additional group of monkeys received daily injections with INTRON®-A at 3105 μg/m^2 as a comparative control. Samples of peripheral blood for determination of PEG-IFN levels were obtained from all monkeys at baseline (immediately prior to dosing on day 1), and at 4, 8, 12, 24, 36, and 48 h after the first dose of PEG-IFN or INTRON®-A. Peripheral blood samples for cumulative exposure were also obtained at these same time points before and after dosing on day 23 of study (week 4), and on day 5 at 0, 4, and 48 h post-dose.

Serum interferon levels were measured both by ELISA, and by specific inhibition of viral cytopathic effect (CPE bioassay). Antibody activity (both total Ig and neutralizing antibody) was determined by enzyme immunoassay (EIA) and the CPE bioassay, respectively. The results from samples obtained on day 1 of dosing, as determined by both assays are presented in Table V, below:

Table V – Toxicokinetic Evaluation of PEG-IFN in Cynomolgus Monkeys – Day 1

A. ELISA Assay for IFN levels

	Mean Value ± % CV			
	PEG-IFN Dose Level			INTRON®-A
T/K Parameter	1414 μg/m^2	4239 μg/m^2	14130 μg/m^2	3105 μg/m^2
C_{max} (IU/ml)	7428 ± 30	22436 ± 49	59249 ± 21	63438 ± 37
T_{max} (h)	4.0 ± 0	6.0 ± 37	7.33 ± 22	4.67 ± 35
$t1/2_{elim}$ (h)	24.6 ± 17	24.9 ± 23[a]	30.1 ± 36	n.a.[b]
$AUC_{(0\text{-}48h)}$ (IU·hr/ml)	201900 ± 20	475600 ± 17[a]	1459000 ± 14	544644 ± 22
$AUC_{(0\text{-}\infty)}$ (IU·hr/ml)	283700 ± 22	675100 ± 17[a]	2220000 ± 23	n.a.[b]

[a] n = 5 animals

[b] n.a. = not amenable to toxicokinetic analysis

B. CPE Bioassay for IFN levels

	Mean Value ± % CV			
	PEG-IFN Dose Level			INTRON®-A
T/K Parameter	1414 μg/m^2	4239 μg/m^2	14130 μg/m^2	3105 μg/m^2
C_{max} (IU/ml)	13200 ± 43	48800 ± 56	89600 ± 22	54400 ± 35
T_{max} (h)	4.0 ± 0	12.7 ± 73	4.67 ± 35	4.67 ± 35
$t1/2_{elim}$ (h)	15.8 ± 34	8.0 ± 20[a]	25.6 ± 37[b]	3.89 ± 37
$AUC_{(0\text{-}48h)}$ (IU·hr/ml)	222400 ± 17	1012800 ± 23	2800000 ± 18	520640 ± 33
$AUC_{(0\text{-}\infty)}$ (IU·hr/ml)	266100 ± 20	1256500 ± --[a]	4285700 ± 34[b]	520830 ± 33

[a] n = 1 animal

[b] n = 5 animals

No interferon activity was detected by either assay in serum samples from the vehicle control (SCH 54031 placebo) treated monkeys at any time point on study. The increases in AUC (both $AUC_{0\text{-}48h}$ and $AUC_{0\text{-}\infty}$) and the levels of C_{max} were increased in a linear manner in proportion to the dose of PEG-IFN administered. However, in all groups treated with PEG-IFN, the C_{max} values obtained by the CPE bioassay were approximately 2-fold higher than those obtained using the ELISA to detect IFN levels. This effect was not observed when serum samples from monkeys treated with INTRON®-A as a comparative control were analyzed for IFN activity in the two assays. At the highest dose of PEG-IFN tested, total exposure to IFN (as determined by both $AUC_{0\text{-}48h}$ and $AUC_{0\text{-}\infty}$) was increased by approximately 5-fold over exposure to INTRON®-A.

At d 5 on study, detectable interferon activity (ranging from 2000 to 22,400 IU/ml) was present in PEG-IFN treated monkeys at time 0 (prior to treatment), confirming that the exposure to the PEG-modified product was prolonged, compared to that achieved with unmodified INTRON®-A. At 4 h after injection of 4239 $\mu g/m^2$ PEG-IFN on study d 5, serum IFN activity was increased by approximately 60% over levels obtained at the same point on d 1, suggesting that bioaccumulation of the agent was occurring following repeat administration. However, by week 4 on study, significant neutralizing antibody to IFN had developed in all monkeys treated with PEG-IFN, as well as those animals injected with INTRON®-A, resulting in decreased levels of detectable IFN in either of the two assays.

In summary, s/c injection of PEG-IFN at doses of 1414, 4239, or 14,132 $\mu g/m^2$ in a repeat-dose toxicity study was associated with dose-related increases in C_{max} and AUC following the first administration, and evidence of bioaccumulation of IFN activity by d 5 (third dose) on study. After 4 weeks of treatment, detection of serum IFN levels was impeded by the development of neutralizing antibody titers.

Study #D-27156. ***SCH54031: Tissue distribution of radioactivity by whole body autoradiography following a single administration of ^{125}I-interferon-α 2b or ^{125}I-PEG$_{12000}$-IFN-α 2b.***

The tissue distribution of ^{125}I-labeled, PEG-IFN or ^{125}I-labeled INTRON®-A was evaluated in male Sprague-Dawley rats following a single, s/c injection. INTRON®-A and PEG-IFN were radiolabeled using N-succinimidyl-p-iodobenzoate (PIB) reagent. Specific activity of the stock solution of ^{125}I-labeled PEG-IFN was 161μCi/ml, with a total protein concentration of 0.236 mg/ml. INTRON®-A specific activity in the stock solution after ^{125}I-labeling was 217 μCi/ml, with a total protein concentration of 0.177 mg/ml. Radiolabeled PEG-IFN was mixed with non-labeled PEG-IFN prior to dosing, to yield a final protein concentration of 0.419 mg/ml and a specific activity of 128 μCi/ml.

Rats were injected at time zero with 172.7 μg each of radiolabeled PEG-IFN, or 50.4 μg/rat labeled IFN, for a dose of approximately 50 μCi ^{125}I activity. Additional groups of rats were treated with Na^{125}I or ^{125}I-PIB as reagent controls. Two rats per time point were euthanized by CO2 inhalation, the body hair removed by shaving, and the rats quick-frozen in a hexane-dry ice bath (-70oC) for approximately 3 to 5 minutes. The animals were then placed in a –40°C freezer to allow evaporation of the hexane. One rat per group was used for tissue analysis; the remainder of the samples was discarded at the end of the study.

Whole-body, sagittal sections (30 μ) were prepared, and two serial sections per rat evaluated by autoradiography for distribution of ^{125}I-activity. One section was evaluated without further processing; the second section was treated with 10% trichloroacetic acid to precipitate any radiolabeled, acid-precipitable proteins and then washed to remove any unbound radioactivity. The sections were then exposed for 24 h to phosphor imaging plates, and the resulting autoradiographs evaluated visually after image transfer to a microcomputer, for distribution of the drug-derived radioactivity.

At 1 h after injection of ^{125}I-INTRON®-A, distribution of radioactivity was highest in the blood, heart, and kidneys, and was present in other tissues, including the liver, gastrointestinal tract, and thyroid. Levels of radiolabel had decreased in most tissues by 4 h after injection, with the exception of the gastrointestinal contents, which had the highest level at this time point and decreased by 24 h post-dose. By 24 h after injection, tissue radioactivity was negligible. There was no distribution of ^{125}I radiolabel into the brain at any time point after ^{125}I-INTRON®-A administration.

A similar pattern of tissue distribution of radioactivity was observed in rats injected with ^{125}I-PEG-IFN, however, the time course of distribution differed from that observed with the unmodified, radiolabeled IFN. Peak tissue radioactivity was observed at 4 and 24 h post-dosing in these animals, and decreased steadily between 24 and 72 h after injection, suggesting a prolonged absorption from the injection site. Radiolabel was detected in the blood (concentrated in the heart) and tissues, especially the kidney, liver, and lung, as well as in the gastrointestinal tract and the bladder. Detectable levels of radiolabel were still present in the blood and tissues at 72 h after injection of ^{125}I-PEG-IFN, indicative of a slower elimination of the drug-derived radioactivity. There was no distribution of ^{125}I radiolabel into the brain at any time point after ^{125}I-PEG-IFN injection.

Autoradiographic analysis of trichloracetic acid-treated sections revealed much lower intensity of distribution of radiolabel than did the untreated sections, suggesting that the majority of radioactivity detected in the tissues was no longer associated with the IFN or PEG-IFN proteins. Radioactivity within the gastrointestinal contents was removed following trichloracetic acid washing of the sections, suggesting that the radiolabel non-specifically bound to food particles within the stomach or gastrointestinal tract.

In summary, tissue-associated radioactivity reached peak levels within 1 h after injection of ^{125}I-INTRON®-A, was widely distributed throughout the body, and was rapidly eliminated via the kidney by 24 h after injection. By contrast, peak levels of tissue radioactivity after ^{125}I-PEG-IFN were not achieved until 4 to 24 h after injection, and a much slower elimination of the agent occurred, with drug-associated radiolabel still detectable in the blood and tissues at 72 h post-dosing. The tissue distribution patterns of radiolabel were qualitatively similar for PEG-IFN and INTRON®-A, with the main sites of distribution being the blood, heart, kidney, liver, and thyroid.

Study #9818: ——————— ; Document ——— . *SCH 54031: Metabolism and excretion of PEG_{12000}[^{125}I]-IFN α-2b following a single subcutaneous dose to male cynomolgus monkeys.*

The disposition of ^{125}I-labeled, SCH54031 PEG-IFN was determined in male cynomolgus monkeys. Three monkeys received treatment with 1413 μg/m^2 radiolabeled PEG-IFN (specific activity, 608 μCi/mg protein) by s/c injection on day 0. Following dosing, the injection site was covered with a gauze pad, slight pressure applied, and the needle removed. The gauze pads were analyzed for radioactivity using a ——————— Auto-gamma counter. Peripheral blood samples for determination of radioactivity were collected at 1, 4, 8, 24, 48, 72, 96, and 168 h after dosing. Serum samples were prepared, and aliquots were analyzed both for radioactivity by gamma scintigraphy, and for metabolite profiles by high performance liquid chromatography.

Urine and fecal samples were collected at block intervals from 0-4, 4-8, and 8-24 hours after dosing, and then every 24 hours until study termination at 168 h. Total weight of each urine sample was recorded, and 20 ml aliquots were stored at –70°C for analysis of metabolite profiles. The remainder of the urine samples was stored at –20°C for analysis of radiolabel by gamma counting. The total weight of each fecal sample was recorded and the materials were transferred to individually labeled plastic containers, and stored at –20°C until analysis by gamma scintigraphy.

For analysis of radioactivity, 0.1 g samples of serum and 0.2 g samples of urine from the 0-4, 4-8, and 8-24 h time points were analyzed directly. Approximately 1 g samples of urine from all later time points were analyzed for each replicate. Fecal samples were homogenized in 2-3 volumes of a 50:50 methanol:water solution, and duplicate, 1 g aliquots removed for analysis by gamma counting.

All samples were analyzed in duplicate for gamma radioactivity in a ——————— Auto-gamma counter for 5 min, or until 1,000,000 counts had accumulated. Any sample below the lower limit of quantitation (100 cpm) was reported as zero. Validation of the radioactivity analyses in the different samples was conducted by spiking control samples of urine, serum, or feces with known quantities of ^{125}I-PEG-IFN. Overall mean recovery in the spiked samples was 100%; therefore, the measured values for radioactivity were not corrected for percent recovery.

Metabolite profiles were determined by high performance, size exclusion chromatography (HPSEC) on selected serum and urine samples, and compared against reference samples of SCH 54031 prepared from the initial dosing solutions. Additional analysis of urine samples was conducted by SDS-PAGE gel electrophoresis, followed by staining with Coomassie blue to visualize the proteins, and overnight exposure to phosphor imaging plates for determination of radiolabel by autoradiography.

Analysis of the serum samples revealed mean, peak radioactivity of 0.344 μg equivalents ^{125}I-PEG-IFN/g serum at 4 h following a single s/c dose, and declining over the duration of the study to a final, mean value of 0.004 mg equivalents/g sample at 168 h post-dosing. The majority of the radioactivity was excreted in the urine (total 69.8 – 91.8% of initial dose) during the first 72 h after injection, with a cumulative, mean total of 93% excreted by study termination at 168 h. Less than 1% of the radiolabel was recovered in the feces over the duration of the study.

Only 8-10% of the radiolabel in the urine was detected following trichloracetic acid precipitation, suggesting that the majority of excreted material was associated with either small peptides, amino acids, or free ^{125}iodine. This was confirmed by analysis of the metabolite profiles of ^{125}I-PEG-IFN by HPSEC, which revealed that the parent material was present in the serum at up to 72 h post-dosing, and was not present in urine at any time point evaluated. Free interferon was not detected in either serum or urine at any time point. At 48, 72, and 96 h post-dosing, the major radiolabeled metabolite detected in serum was a peak with the characteristics of a PEG-IFN dimer. This peak had previously been described as a possible impurity in the test article; however, its appearance on the HPSEC chromatogram increased with time after dosing, suggesting that it was one of the major circulating forms of PEG-IFN after injection.

In summary, following a single, s/c injection of ^{125}I-PEG-IFN to male cynomolgus monkeys, serum radioactivity concentrations were found to peak 4 h after treatment, and remain detectable until 72 h post-dosing. Greater than 90% of the radiolabel was excreted in the urine, with neither free IFN, nor the parent, PEG-IFN material detectable. These findings are similar to those previously obtained with SCH 30500 (INTRON®-A).

PRECLINICAL TOXICOLOGY:

Mutagenicity Study Summary:

1. *Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay of SCH 54031 (PEG_{12000}IFN alfa 2B). Study #96240 ( ). SCH 54031 (lot #33208-157), 12.5, 25, 50, 100, or 175 µg/plate, or placebo control (lot #35923-001), with or without Aroclor™-activated, rat liver microsomes (S-9 fraction); GLP; 9/5 – 12/23/96; . **Volume 11, pp. 1-81 (Reference 10).**

2. Chromosome aberration study of SCH 54031 (PEG_{12000}IFN alfa-2B) in human peripheral blood lymphocytes. Study #96241). SCH 54031 (lot #33208-157), 0.55, 1.1, 2.19, 4.38, 8.75, 17.5, or 35 µg/ml, or placebo control (lot #35923-001), with or without Aroclor™ 1254-activated, rat liver microsomes (S-9 fraction); GLP; 8/2 – 10/3/96; **Volume 11, pp. 1-67 (Reference 11).**

3. Mouse bone marrow erythrocyte micronucleus study of SCH 54031. Study #98450. Crl:CD-1® [ICR]BR VAF/Plus® mice; 6/sex/group, weight range 24.0-32.1 g (male), 20.5-25.7 g (female); 0.9% sterile saline, lot #35-425-DK, 50 ml/kg, i/p; 1.88, 3.75, 7.5, 15, 30 mg/kg/d PEG-IFN (lot #38101-016), x 2 days, i/p; cyclophosphamide (lot #073H0846, Sigma), 50 mg/kg, i/p; GLP; 10/12 – 12/11/98; Safety Evaluation Center, Schering-Plough Research Institute, Lafayette, NJ. **Volume 11, pp. 1-64 (Reference 12).**

4. Bacterial mutagenicity study of SCH 215600. Study #98520. SCH 215600 PEG (lot #), 0, 313, 625, 1250, 2500, 5000 µg/plate, with or without Aroclor™ 1254-activated, rat liver microsomes (S-9 fraction); GLP; 11/11/98 – 2/12/99; Schering-Plough Research Institute, Lafayette, NJ. **Volume 23, pp. 1-57 (Reference 26).**

5. Chromosomal aberration study of SCH 215600 (methoxypolyethylene $glycol_{12000}$) in human peripheral blood lymphocytes. Study #98518). SCH 215600

(lot #8425-115), 2.38, 4.75, 9.5, 19, 38, 76, 152, 605, 1210, 2410, 2500, 3000, 4000, 4810, or 5000 µg/ml, or vehicle control (sterile water for injection), with or without Aroclor™1254-activated, rat liver microsomes (S-9 fraction); GLP; 11/11 – 12/15/98; ———— ———— **Volume 23, pp. 1-73 (Reference 27).**

Mutagenicity Study Review:

Study #96240 ———— ***Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay of SCH 54031 (PEG_{12000}IFN alfa 2B).***

Cultures of TA98, TA 100, TA 1535, TA97a, and TA102 test strains of *Salmonella typhimurium* and *Escherichia coli* tester strain WP2*uvr*A were incubated in triplicate at 37°C for 72 hrs with PEG-IFN at multiple concentrations ranging from 12.5 to 175 µg/plate. The PEG-IFN preparation was diluted in the placebo for PEG-IFN, and the PEG-IFN placebo was also used as the vehicle control for the experiment. The highest concentration of PEG-IFN tested (175 µg/plate) was the highest level that could be feasibly tested in this system, based on reconstitution vial concentrations. Positive control chemicals used were 2-aminoanthracene (2.5-25 µg/plate), 2-nitrofluorene (1.0 µg/plate), sodium azide (2 µg/plate), ICR-191 (2.0 µg/plate, tester strain TA97a only), and 4-nitroquinoline-N-oxide (1.0 µg/plate, *E. coli* WP2*uvr*A test strain only).

Range-finding studies were initially conducted to determine the cytotoxic (growth-inhibitory) effect of PEG-IFN on the test system. *Salmonella typhimurium* tester strain TA 100 and *E. coli* tester strain WP2*uvr*A were incubated in increasing concentrations of PEG-IFN (0.01 to 175 µg/plate, by half-log dilutions), both in the presence and the absence of metabolic activation by rat liver microsomes. No cytotoxicity of PEG-IFN to either of the tester strains was observed in the range-finding assay, at concentrations of up to 175 µg/plate.

The mutagenicity assay was performed using the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA97a, and TA102, and the *E. coli* test strain WP2*uvr*A. Five doses of PEG-IFN, ranging from 12.5 to 175 µg/plate were tested in the assay, in the presence or absence of Aroclor™ activated rat liver microsomes. PEG-IFN exhibited no toxicity to any of the test strains at doses as high as 175 µg/plate. All of the test strains treated with PEG-IFN exhibited mean reversion frequencies (number of histidine$^+$ revertant colonies above the control incidence) similar to the vehicle (PEG-IFN placebo) controls, with or without S9 activation. There was no evidence of dose-related effects on reverse mutation up to the highest concentration of PEG-IFN tested. Each positive control produced a marked mutagenic response in the appropriate tester strain. These results were confirmed in three additional assays, using the same range of concentrations of PEG-IFN and positive and negative controls, with or without metabolic activation.

In summary, PEG-IFN exhibited no evidence of mutagenic potential in five tester strains of *Salmonella typhimurium*, and in *E. coli* strain WP2*uvr*A, using the standard Ames microbial mutagenicity plate incorporation tests.

Study #96241 [redacted] ***Chromosome aberration study of SCH 54031 (PEG12000IFN alfa-2B) in human peripheral blood lymphocytes.***

The clastogenic potential of PEG-IFN was assessed in an *in vitro* assay using mitogen-stimulated, primary human peripheral blood lymphocytes in the presence and absence of Aroclor™-induced rat liver microsomes as an exogenous source of metabolic activation. Duplicate cultures of phytohemagglutinin-stimulated cells obtained from one male and one female human donor were treated with PEG-IFN at concentrations ranging from 0.55 to 35 μg/ml for 3 to 48 hours in the absence of metabolic activation, and for 3 hours when rat liver S9 microsomes were included in the preparation. The highest concentration of PEG-IFN tested was the highest level that could feasibly be used in this system, in a total volume of 10 ml. Placebo for PEG-IFN was used as the vehicle control, while cells suspended only in culture medium (RPMI 1640 medium plus 15% fetal bovine serum and antibiotics) were used as the negative control. Mitomycin C (1.5 μg/ml) and 30 μg/ml cyclophosphamide were used as positive control agents in non-activated and activated phases of the study, respectively.

Following exposure to the test articles, the cells were incubated for 2 hours in colchicine, harvested at approximately 24 and 48 hours after incubation with PEG-IFN, stained with Giemsa stain, and evaluated microscopically for evidence of chromosomal breaks or translocations, polyploidy, and reduction in mitotic indices.

In the absence of metabolic activation, no reduction in mitotic indices, nor increases in cells with chromosomal aberrations, polyploidy, or endoreduplication were observed in the lymphocytes from the male donor at any concentration of PEG-IFN tested after a 24 hour exposure. After 48 hours, a dose-related reduction in mitotic index, with decreases of 11 and 17% as compared to the vehicle control after incubation in 17.5 and 35 μg/ml PEG-IFN, respectively, was observed in the cells from this donor. Similar findings were observed at the 24, but not the 48 hour time point in cells from the female donor, however, there was no apparent dose-relationship, and at 48 hours there were no effects on mitotic index at any concentration of PEG-IFN tested in this donor's cells. However, there was no increase in the number of cells from either donor with chromosomal aberrations at any dose at this time point, suggesting a direct, cytotoxic effect of PEG-IFN on cultured lymphocytes.

Mitotic indices were also decreased, although with no dose-relationship, when lymphocytes from either donor were incubated with increasing concentrations of PEG-IFN in the presence of metabolic activation by rat liver microsomal S-9 fraction. Reductions in mitotic index of 55%, 25%, and 8%, as compared to vehicle control were observed at the 24 hour harvest after a 3 hour incubation with 2.19, 4.38, and 17.5 μg/ml PEG-IFN, respectively, in cells from the female donor. In the male donor at the same time point, reductions in mitotic indices ranging from 9 to 30% as compared to vehicle control were observed at all doses of PEG-IFN tested, with no apparent relationship to the concentration of the test article. Similar reductions in mitotic indices were observed in cells from both donors at the 48 hour harvest, suggesting that PEG-IFN was inducing the changes by a direct, cytotoxic effect on these cells that was unrelated to any effect on proliferation.

The percentage of cells with chromosomal aberrations ranged from 0 to 2% for any of the PEG-IFN concentrations tested, either with or without S-9 metabolic activation. Negative control cultures had total aberrant cell indices of 1-2%, regardless of whether S9 fraction was present.

Aberrations included chromosomal gaps, breaks, translocations, polyploidy, or endoreduplication, and occurred at approximately equal incidence in both the negative and vehicle controls, as well as in the PEG-IFN treated groups. The positive controls cyclophosphamide and mitomycin C produced the expected clastogenic responses in cells from both donors, with the total percentage of aberrant cells in both of the positive control groups ranging from 2 to 24% of the metaphase cells examined. These results are in the range of values expected for mitomycin C and cyclophosphamide under the conditions of this assay, confirming the sensitivity of the test system.

In summary, PEG-IFN showed no evidence of clastogenic potential in this *in vitro* human peripheral blood lymphocyte chromosomal aberration assay at up to the highest feasible concentration (35 μg/ml) which could be evaluated.

Study #98450. ***Mouse bone marrow erythrocyte micronucleus study of SCH 54031.***

The potential of PEG-IFN to induce chromosomal damage was evaluated *in vivo* using the mouse bone marrow micronucleus assay. This test is used to screen agents that cause chromosomal damage, manifested by acentric chromatids and chromosome fragments, which are retained by the daughter cells during mitosis as secondary nuclei. The presence of micronuclei in the cell cytoplasm constitutes evidence that the DNA has undergone some type of damage, in response to the test article.

An initial dose-ranging study was conducted in mice to determine the toxicity of the test article. Six mice per sex were treated by i/p injection with the negative control (0.9% sterile saline), or 1.88, 3.75, 7.5, 15, and 30 mg/kg PEG-IFN on days 1 and 2. Three mice per sex were sacrificed at 24 hours after the second injection of PEG-IFN, and the remaining animals were held for four days after initiation of dosing for observation of toxicity. Bone marrow erythrocyte smears were prepared from femoral bone marrow samples, fixed and stained with acridine orange for evaluation of chromosomal abnormalities by fluorescent microscopy.

No clinical signs of toxicity, nor evidence of increased nucleated cells in bone marrow smears was observed at any dose of PEG-IFN tested, as compared to the saline control group. The results of the range-finding assay permitted the use of a decreased number of doses of PEG-IFN for the definitive testing.

Six mice per sex per group were used for the definitive assay, and dosed daily for two consecutive days by i/p injection with sterile saline, 7.5, 15, or 30 mg/kg PEG-IFN. An additional group of six mice per sex was dosed with 50 mg/kg/day cyclophosphamide, i/p in the first assay, and 30 mg/kg cyclophosphamide in the second study as a positive control. In the first study, mice were sacrificed 24 hours after the second dose of the test articles; in the second study, animals were euthanized 48 hours after the second dose of test article. Bone marrow smears were prepared as described above for the dose-ranging assay. Micronucleated erythrocytes were scored by fluorescent microscopy; for each mouse, approximated 2000 polychromatic nucleated erythrocytes (PCE) were evaluated for the presence of micronuclei. Micronucleated, normochromatic erythrocytes (NCE) were estimated based on the number of micronucleated NCE counted and an

estimated total number of NCE, according to the methods of Hart and Engbert-Pedersen[2], to determine the PCE/NCE ratio for each dose group.

The number of micronuclei present in the saline control cells ranged from 1/2000 to 6/2000, and from 0/2000 to 6/2000 in cells from the PEG-IFN-treated mice at both time points after injection. There were no statistically significant increases in the incidence of micronuclei formation as compared to the saline control group for any of the groups of mice treated with PEG-IFN. By contrast, the number of micronucleated cells at 24 hours after dosing in the animals treated with 50 mg/kg cyclophosphamide ranged from 33 to 83 per 2000 PCE evaluated. The number of micronucleated cells observed at 48 hours after 30 mg/kg cyclophosphamide ranged from 15 to 52 cells per 2000 PCE evaluated, confirming the sensitivity of the assay. There were no remarkable differences in the mean values for the PCE/NCE ratios in any of the groups of PEG-IFN treated animals, as compared to cells obtained from mice treated with the saline control.

In summary, the results of this assay demonstrate that under the conditions employed, PEG-IFN at doses of up to 30 mg/kg/d, i/p did not induce any statistically significant changes in the incidence of micronucleated bone marrow cells, suggesting that it is not clastogenic after *in vivo* exposure.

Comment: The studies conducted using the *Salmonella typhimurium* and *E. coli* bacterial mutagenesis assays (Ames test) are inappropriate for protein biotherapeutics. Similarly, the *in vitro* assay using cultured human peripheral blood lymphocytes and the *in vivo* micronucleus assay in mice will not provide relevant information regarding the clastogenic potential of PEG-IFN. These assays are designed to detect mutagenic effects of small molecule drugs, chemicals, and environmental agents that cause direct damage to DNA molecules.

Study #98520. ***Bacterial mutagenicity study of SCH 215600.***

Cultures of TA98, TA 100, TA 1535, TA97a, and TA102 test strains of *Salmonella typhimurium* and *Escherichia coli* tester strain WP2*uvr*A were incubated in triplicate at 37°C for 72 hrs with SCH 215600 PEG at multiple concentrations ranging from 313 to 5000 µg/plate. The PEG preparation was diluted sterile water for injection, which was also used as the vehicle (solvent) control for the experiment. Positive control chemicals used were 2-aminoanthracene (2.5-20 µg/plate), 2-nitrofluorene (5 µg/plate), sodium azide (5 µg/plate), 9-aminoacridine (75 µg/plate, tester strain TA97a only), cumene hydroperoxide (100 mg/plate, tester strain TA 102 only), and N-ethyl-N'-nitro-N-nitrosoguanidine (2.0 µg/plate, *E. coli* WP2*uvr*A test strain only).

No range-finding studies were performed. The initial assay was conducted using the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA97a, and TA102, and the *E. coli* test strain WP2*uvr*A, to determine both the potential to induce mutations, as well as to evaluate the cytotoxic (growth-inhibitory) effect of SCH 215600 PEG on the test system. All five *Salmonella typhimurium* tester strains and *E. coli* tester strain WP2*uvr*A were incubated in increasing concentrations of SCH 215600 (313 to 5000 µg/plate, by two-fold dilutions), both in the presence and the absence of metabolic activation by rat liver microsomes. No increased incidence in histidine$^+$ revertants was observed over the solvent control group, and there was no detectable

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[2] Hart, J.W. and H. Engbert-Pedersen. 1983. Statistics of mouse bone-marrow micronucleus test: Counting, distribution, and evaluation of results, *Mutat. Res.*, **111**:195-207.

cytotoxicity of PEG to any of the tester strains was observed in the range-finding assay, at concentrations of up to 5000 µg/plate.

The mutagenicity assay was confirmed in a second assay, using all five *Salmonella typhimurium* tester strains and the *E. coli* test strain WP2*uvr*A. Five doses of SCH 215600 PEG, ranging from 313 to 5000 µg/plate were tested in the assay, in the presence or absence of Aroclor™ activated rat liver microsomes. In the second assay, SCH 215600 PEG exhibited no toxicity to any of the test strains at doses as high as 5000 µg/plate. All of the test strains treated with PEG exhibited mean reversion frequencies (number of histidine$^+$ revertant colonies above the control incidence) similar to the solvent (sterile water for injection) controls, with or without S9 activation. There was no evidence of dose-related effects on reverse mutation up to the highest concentration of SCH 215600 tested. Each positive control produced a marked mutagenic response in the appropriate tester strain.

In summary, SCH 215600 methoxy-polyethylene glycol exhibited no evidence of mutagenic potential in five tester strains of *Salmonella typhimurium*, and in *E. coli* strain WP2*uvr*A, using the standard Ames microbial mutagenicity plate incorporation tests.

Study #98518 (———————). *Chromosome aberration study of SCH 215600 (methoxypolyethylene glycol$_{12000}$) in human peripheral blood lymphocytes.*

The ability of SCH 215600 methoxy-polyethylene glycol (PEG) to induce chromosomal aberrations was assessed in an *in vitro* assay using mitogen-stimulated, primary human peripheral blood lymphocytes in the presence and absence of Aroclor™-induced rat liver microsomes as an exogenous source of metabolic activation. Duplicate cultures of phytohemagglutinin-stimulated cells obtained from one male and one female human donor were treated with SCH 215600 PEG for 4 or 19 hours, respectively, in the absence of metabolic activation, at concentrations ranging from 2.38 to 5000 µg/ml. Cells from both donors were treated with PEG for 4 hours when rat liver S9 microsomes were included in the preparation. The initial study used peripheral blood lymphocytes from a male donor, and concentrations of SCH 215600 of 2.38, 4.75, 9.5, 19, 38, 76, 153, 605, 1210, 2410, and 4820 µg/ml in a volume of 10 ml. Because of the high viscosity of the PEG solution at the 4820 µg/ml concentration, the volume of test medium was increased to 20 ml for the second, confirmatory assay using lymphocytes from a female donor, and test concentrations of 626, 1250, 2500, and 5000 mg/ml SCH 215600 PEG. Sterile water for injection was added to cells suspended in culture medium (RPMI 1640 medium plus 15% fetal bovine serum and antibiotics) as the vehicle control, and cells suspended only in culture medium served as the negative control. Mitomycin C (0.1-1.5 mg/ml) and 25-75 mg/ml cyclophosphamide were used as positive control agents in the non-activated and activated phases of the study, respectively.

Following exposure to the test articles, the cells were incubated for 2 hours in colchicine, harvested at approximately 22 hours after treatment with SCH 215600, stained with Giemsa stain, and evaluated microscopically for evidence of chromosomal breaks or translocations, polyploidy, and reduction in mitotic indices.

In the first study, mitotic indices were not evaluated for cells treated with 2.38, 4.75, 9.5, or 19 µg/ml SCH 215600. In the absence of metabolic activation, no reductions in mitotic indices were observed in cells treated with 38, 76, 152, 605, 1210, 2410, or 4280 µg/ml PEG, as compared to

cells treated with the vehicle control. A 205 decrease in mitotic index was noted from cells treated with 303 mg/ml PEG for 4 hours as compared to the control cultures; however, since this effect was not observed at any other dose of SCH 215600, it was considered to be a spurious finding. There were no increases in cells with chromosomal aberrations, polyploidy, endoreduplication, or chromosomal aberrations at any of the doses of SCH 215600 PEG evaluated.

In the second study, cells from the female donor were exposed to SCH 215600 for 19 hours in the absence of metabolic activation, then harvested approximately 4 hours later. No reductions in mitotic indices were noted after exposure to 1250, 2000, 2500, 3000, 4000, or 50000 μg/ml SCH 215600; the lower doses of PEG-treated cells were not evaluated. Chromosomal aberrations were analyzed only for the cultures exposed to 2500, 3000, 4000, or 5000 μg/ml SCH 215600. There were no statistically significant increases in cells with polyploidy, endoreduplication, or chromosomal aberrations at any of the doses evaluated. By contrast, a 27% mean reduction in mitotic index, and chromatid and/or chromosome breaks, gaps, and exchanges were observed in 5 to 25% of the cells treated with 0.3 μg/ml mitomycin C as a positive control.

In the presence of metabolic activation by rat liver microsomes, there were no reductions in mitotic indices observed in cells from the male donor exposed to SCH 215600, as compared to the vehicle control. In the second study, a 31% reduction in mitotic index was observed in cells exposed to 626 μg/ml PEG in the presence of metabolic activation by rat liver S 9 fraction, as compared to cells exposed to the vehicle control and rat liver microsomes. In both studies, chromosomal aberrations were evaluated only for samples treated with ≥ 605 μg/ml SCH 215600 PEG; no statistically significant increases in the number of cells with polyploidy, endoreduplication, or chromosomal aberrations were observed at any dose level of SCH 215600, as compared to the vehicle or negative control groups. By contrast, treatment with 50 μg/ml cyclophosphamide in the presence of metabolic activation produced reductions in mitotic index of 78 to 92%, and chromosomal and/or chromatid breaks, gaps, and translocations in 8 to 38% of the cells, confirming the sensitivity of the assay.

In summary, SCH 215600 PEG showed no evidence of clastogenic potential in this *in vitro* human peripheral blood lymphocyte chromosomal aberration assay at up to the highest feasible concentration (5000 μg/ml) which could be evaluated.

Toxicology Study Summary:

1. Acute subcutaneous toxicity study of SCH 54031 (PEG_{12000}-IFN-α 2b) in mice. Study #95026 (Report #P-6090). Crl:CD-1®(ICR)BR VAF/Plus™ mice, 5/sex, six weeks old, weight range 21.4-24 g (♂), 17.5-22.7 g (♀); vehicle control (SCH 54031 placebo, lot #35923-001, composition nit specified) or 60,410 μg/m^2 SCH54031, lot #33208-157; GLP; 4/11 - 7/21/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 7, pp. 1-39 (Reference 1).**

2. Acute intravenous toxicity study of SCH 54031 (PEG_{12000}-IFN-α 2b) in mice. Study #95027 (Report #P-6091). Crl:CD-1®(ICR)BR VAF/Plus™ mice, 5/sex, six weeks old, weight range 21.1-25.3 g (♂), 17.0-22.4 g (♀); vehicle control (SCH 54031 placebo, lot #35923-001, composition not specified), or 30,205 μg/m^2 SCH 54031, lot #33208-157; GLP; 4/11-7/21/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 7, pp. 1-39 (Reference 2).**

3. Acute subcutaneous toxicity study of SCH 54031 (PEG_{12000}-IFN-α 2b) in rats. Study #95024 (Report #P-6088). Crl:CD-1®(SD)BR VAF/Plus™ rats, 5/sex/group, six weeks old, weight range 149.5-167.8 g (♂), 124.9-148.2 g (♀); vehicle control (SCH 54031 placebo, lot #35923-001, composition not specified) or 56,850 μg/m^2 SCH54031, lot #33208-157; GLP; 4/12 – 7/21/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 7, pp. 1-40 (Reference 3).**

4. Acute intravenous toxicity study of SCH 54031 (PEG_{12000}-IFN-α 2b) in rats. Study #95025 (Report #P-6089). Crl:CD-1®(SD)BR VAF/Plus™ rats (weight range 149.8-171.1 g (♂), 127.1-142.6 g (♀), 5/sex; vehicle control (placebo), lot #35923-001 or 56,850 μg/m^2 SCH54031, lot #33208-157; GLP; 4/12 – 7/21/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 7, pp. 1-40 (Reference 4).**

5. Subcutaneous single dose tolerance study of SCH54031 (PEG_{12000}-IFN-α 2b) in cynomolgus monkeys. Study #95028 (Report #P-6135). *Macaca fasicularis*, 2/sex/group, young adult animals, weight range 2.8-3.6 kg (♂), 2.7-3.4 kg (♀); 29,435, 58,861, 117,721 μg/m^2 SCH54031, lot #33208-157; GLP; 4/13-11/17/95; Schering-Plough Research Institute, Lafayette, NJ. (**Please note** - is same study as pharmacokinetics study #4, Schering Study #P-6135). **Volume 7, pp. 1-185 (Reference 5).**

6. One-month subcutaneous toxicity study of SCH 54031 (PEG_{12000}-IFN-α 2b) solution in cynomolgus monkeys. Study #94081 (Schering Report #P-6136). *Macaca fasicularis* (weight range 2.3-3.7 kg, ♂; 2.2-3.7 kg, ♀), 3/sex/group; vehicle control (IFN placebo, lot #35293-001), 1414, 4239, 14130 μg/m^2/dose SCH54031, lot #33208-157, or 3105 μg/m^2 INTRON®-A, lot #33208-155-03; GLP; 4/4-11/7/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 8, pp. 1-308 and Volume 9, pp. 309-527 (Reference 6).** (**Please note** - is same study as pharmacokinetics study #5, Schering Report #P-6151).

7. Pain on injection study of a parenteral formulation of SCH 54031 (PEG_{12000}-IFN-α 2b) in rats. Study #95029 (Report #P-6102). Crl:CD-1®(SD)BR VAF/Plus™ rats (4 weeks old, weight range 44.3-77.0 g), 10 ♀/group; subplantar injection of 0.1 ml into right rear paw; saline control (0.9% NaCl solution, lot #88-478-DK), vehicle control (IFN placebo, lot #35923-001); SCH54031, lot #33208-157, 35 μg/rat; INTRON®-A, lot #33208-155-03, 19.2 μg/rat; Mefoxin® (positive control), lot #35923-020, 40 mg/rat; GLP; 5/4 – 9/26/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 10, pp. 1-133 (Reference 7).**

8. Acute subcutaneous irritation study of a new parenteral formulation of SCH 54031 (PEG_{12000}-IFN-α 2b) in rats. Study #95030 (Report #P-6082). Crl:CD-1®(SD)BR VAF/Plus™ rats (8 weeks old, weight range 178-272 g), 5 ♂/dose/time point; single s/c injection of 0.3 ml; saline control (0.9% NaCl solution, lot #88-478-DK), vehicle control (IFN placebo, lot #35923-001); SCH54031, lot #33208-157, 105 μg/rat; INTRON®-A, lot #33208-155-03, 57.6 μg/rat; Mefoxin® (positive control), lot #35923-020, 120 mg/rat; 24, 48, 96 h sacrifices; GLP; 4/24 - 4/28/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 10, pp. 1-141 (Reference 8).**

9. Muscle irritation study of a parenteral formulation of SCH 54031 (PEG_{12000}-IFN-α 2b) in rabbits. Study #95031 (Report #P-6083). Hra:(NZW)SPF rabbits (10 months old, weight range 3.3-4.3 kg), 4 ♂/group/time point; single i/m injection of 1.0 ml; saline control (0.9%

NaCl solution, lot #88-478-DK), vehicle control (IFN placebo, lot #35923-001); SCH54031, lot #33208-157, 350 µg/rabbit; INTRON®-A, lot #33208-155-03, 192 µg/rabbit; Mefoxin® (positive control), lot #35923-020, 400 mg/rabbit; 24, 72, 168 h sacrifices; GLP; 4/18 – 4/25/95; Schering-Plough Research Institute, Lafayette, NJ. **Volume 10, pp. 1-126 (Reference 9).**

10. Acute toxicity study of Sch 30500 (alpha-2 INTERFERON) in Rhesus monkeys. Study Report ——. *Macaca mulatta*, 4/sex/group, weight range 1.95 – 3.6 kg (age range 2-3 years old); diluent (agent not specified), 130, 260 x 10^6 IU/kg, i/m; 260 IU/kg, i/v SCH 30500 (INTRON®-A, lot #mf 266); GLP; final study report 5/13/82; —— **Volume 11, pp. 1-28 (Reference 13).**

11. One-month intramuscular toxicity study of SCH 30500 (α2-interferon) in the rat. Study #80094 (Report #P-4782). Charles River CD® rats, 15/sex/group, seven weeks old, weight range 208-234 g (♂), 130-156 g (♀); vehicle (phosphate buffered saline, pH 7.2, lot #12915-087), 1.1 MIU SCH 30500/kg/day, i/m (INTRON®-A, lot #12888-078, specific activity 4.1 x 10^7 IU/mg protein); GLP; 6/1 – 8/14/81; Schering Corporation, Lafayette, NJ. **Volume 12, pp. 1-237 (Reference 14).**

12. One-month intramuscular toxicity study of SCH 30500 (α2-interferon) in the monkey. Study #80093 (Report #P-4783). Cynomolgus monkeys (*Macaca fasicularis*), 3/sex/group, approximately 3 to 4 years old, weight range 3.4-4.6 kg (♂), 2.6-3.2 kg (♀); vehicle (phosphate buffered saline, pH 7.2, lot #12915-087), 1.1 MIU SCH 30500/kg/day, i/m (INTRON®-A, lot #12888-078, specific activity 4.1 x 10^7 IU/mg protein); GLP; 6/3 – 8/14/81; Schering Corporation, Lafayette, NJ. **Volume 12, pp. 1-117 (Reference 15).**

13. Three-month intramuscular toxicity study of SCH 30500 (α2-interferon) in monkeys. Cynomolgus monkeys (*Macaca fasicularis*), 4/sex/group, age 32-57 months, weight range 1.9-5.0 kg (♂), 1.7-3.0 kg (♀); vehicle control (sterile, 0.9% saline, lot #63-616-DK), 4, 20, 100 MIU/kg/day SCH 30500 (INTRON®-A, lots #16931-062-K, 16931-019-K, #16931-070-K) x 91-92 days; GLP; 10/11/84 – 7/30/85; Schering Corporation, Lafayette, NJ. **Volume 13, pp. 1-396 (Reference 16).**

14. Reproductive toxicity of SCH 30500 in Rhesus monkeys (*Macaca mulatta*). Study #85026 —— Study #SCH01). Twelve pregnant ♀/group, age range 4-18 years, weight range 4-10 kg; vehicle control (sterile, 0.15% saline, lot #B83022), 7.5, 15, 30 MIU/kg/day SCH 30500 (INTRON®-A, lot #16931-143K, #16931-141K, and #16931-064K), i/m, GD20-GD80; GLP; 10/20/85 – 11/25/86 —— **Volume 14, pp. 1-72 (Reference 17).**

15. Acute subcutaneous toxicity study of SCH215600 in mice. Study #98448 (— Study —— —— . Crl:CD®-1 [ICR]Br mice, seven weeks old, weight range 27-33 g (♂), 24-29 g (♀); vehicle (SCH 215600 placebo, lot #39554-065), 6480 µg/m^2 SCH 215600 (PEG, lot #39554-066), s/c; GLP; 12/10/98 – 9/10/99; —— . **Volume 18, pp. 1-58 (Reference 18).**

16. Acute intravenous toxicity study of SCH215600 in mice. Study #98449 —— Study —— —— Crl:CD®-1 [ICR]Br mice, seven weeks old, weight range 26-30 g (♂), 24-25 g (♀);

vehicle (SCH 215600 placebo, lot #39554-065), 6480 μg/m^2 SCH 215600 (PEG, lot #39554-066), i/v; GLP; 12/4/98 – 9/10/99; **Volume 18, pp. 1-55 (Reference 19).**

17. Acute subcutaneous toxicity study of SCH215600 in rats. Study #98446 Study Crl:CD®-1 [SD] IGS BR rats, six to seven weeks old, weight range 148-166 g (♂), 133-153 g (♀); vehicle (SCH 215600 placebo, lot #39554-065), 6480 μg/m^2 SCH 215600 (PEG, lot #39554-066), s/c; GLP; 12/3/98 – 9/10/99; **Volume 18, pp. 1-60 (Reference 20).**

18. Acute intravenous toxicity study of SCH215600 in rats. Study #98447 Study Crl:CD®-1 [SD] IGS BR rats, six to seven weeks old, weight range 143-167 g (♂), 133-148 g (♀); vehicle (SCH 215600 placebo, lot #39554-065), 6480 μg/m^2 SCH 215600 (PEG, lot #39554-066), i/v; GLP; 12/3/98 – 9/10/99; **Volume 18, pp. 1-60 (Reference 21).**

19. A 13-week subcutaneous toxicity study of SCH 215600 in rats. Study #SN98222 Study . Crl:CD®(SD)IGS BR rats, 10-15/sex/group, approximately 6-7 weeks old, weight range 185-231 g (♂), 136-176 g (♀); vehicle control 0.9% sterile sodium chloride for injection, U.S.P., lots #C94882, C397331, and C403428), placebo control (SCH 215600 placebo, lot #39554-03; agent not specified), 45.5, 455, 1138 μg/m^2 SCH 215600 (lots #39554-032 and #39554-033), s/c, twice weekly x 13 weeks plus 4 week, treatment-free recovery period in placebo control and high-dose groups; GLP; 9/18/98 – 10/13/99; **Volumes 15, 16, and 17 (Reference 22).**

20. A 13-week subcutaneous toxicity study of SCH 215600 in cynomolgus monkeys. Study #SN98223 Study . *Macaca fasicularis*, 3-5/sex/group, approximately 3.5-5.5 years old, weight range 2.4-3.3 kg (♂), 2.1-2.6 kg (♀); vehicle control 0.9% sterile sodium chloride for injection, U.S.P., lots #C393280, #C391581, C397331, and C403428), placebo control (SCH 215600 placebo, lot #39554-031; agent not specified), 45.5, 455, 1138 μg/m^2/dose SCH 215600 (lots #39554-043, #39554-033, and #39554-066), s/c, twice weekly x 13 weeks plus 4 week, treatment-free recovery period in placebo control and high-dose groups; GLP; 10/2/98 – 10/13/99; **Volumes 18, 19, 20, and 21 (Reference 23).**

21. Embryo-fetal developmental toxicity study of SCH 215600 by subcutaneous injection in rats. Study #SN 98459 Study . Crl:CD®(SD)IGS BR rats, 25 mated ♀/group, approximately 13 weeks old, weight range 255-321 g; placebo control (SCH 215600 placebo, lot #39554-031; composition not specified), 113, 400, 800 μg/m^2 SCH 215600 (lots #39554-032 and #39554-033), s/c, days 6-17 after mating; GLP; 8/25/98 – 5/26/99; **Volume 22, pp. 1-324 (Reference 24).**

22. Embryo-fetal developmental toxicity study of SCH 215600 by subcutaneous injection in rabbits. Study #98291 (Report #P-7028). Hra:[NZW]SPF rabbits, 20 mated ♀/group, 6 to 7 months old, weight range 3.12-4.09 kg; placebo control (SCH 215600 placebo, lot #39554-031; composition not specified), 113, 400, 800 μg/m^2 SCH 215600 (lot #39554-033), s/c, days 7-19 after mating; GLP; 8/24/98 – 4/8/99; Schering-Plough Research Institute, Lafayette, NJ **Volume 23, pp. 1-194 (Reference 25).**

23. Effect of SCH 54031 (PEG_{12000}-IFN) on the menstrual cycle and estradiol and progesterone levels in cynomolgus monkeys. Study #99331. *Macaca fasicularis*, 4 (control) or 7 non-pregnant ♀/group, weight range 2.1 to 4.8 kg; vehicle control (0.9% sterile saline for injection, lot #46-098-DK), 52, 262, 4239 μg/m^2 SCH 54031 (lots #8-IQC-101 and #9-IQD-101), or 3105 μg/m^2 INTRON®-A (lot #8-IFD-001), s/q, q.o.d. for duration of one menstrual cycle or 45 days, whichever longer; GLP; 9/29/99 – 6/1/2000; Safety Evaluation Center, Schering-Plough Research Institute, Lafayette, NJ. **Supplemental volumes I and II, received 6/13/2000.**

Toxicology Review:

Study #95026 (Report #P-6090). ***Acute subcutaneous toxicity study of SCH 54031 (PEG_{12000}-IFN alfa-2b) in mice.***

The acute toxicity of PEG-IFN was evaluated in ICR mice after a single, s/c injection of either vehicle control (SCH 54031 placebo) or 60,410 μg/m^2 SCH 54031 PEG-IFN. The mice were observed immediately after treatment and at 0.25, 0.5, 1, 3, and 5 hours post-dosing for clinical signs of toxicity, then once daily for an additional 14 days. Body weights were determined prior to dosing on day 1, then on days 8 and 15 (prior to sacrifice). Food consumption was recorded daily. Following terminal sacrifice, animals underwent full necropsy and evaluation of gross pathologic changes, including organ weights and morphologic changes. No histopathologic evaluation of tissues was performed.

There were no overt signs of clinical toxicity noted in animals from either the placebo control or the PEG-IFN treated groups. Body weight gains were not appreciably different between the two groups, and there were no changes in food consumption in the mice treated with SCH 54031 as compared to the group injected with the placebo control. At necropsy, no gross pathologic lesions were observed in any animals in either the control or treatment groups that were related to the test article. A single finding of ecchymotic hemorrhage in the lung in one female mouse in the group treated with PEG-IFN (animal #19F) was considered incidental to treatment with the test article.

In summary, treatment of outbred, ICR mice with a single, s/c injection of SCH 54031 PEG-IFN was not associated with any overt signs of toxicity. The NOAEL for PEG-IFN in this species is > 60, 410 μg/m^2 by this route of administration.

Study #95027 (Report #P-6091). ***Acute intravenous toxicity study of SCH 54031 (PEG_{12000}-IFN alfa-2b) in mice.***

The acute toxicity of PEG-IFN was evaluated in ICR mice after a single, i/v injection of either vehicle control (SCH 54031 placebo) or 60,410 μg/m^2 SCH 54031 PEG-IFN. The mice were observed immediately after treatment and at 0.25, 0.5, 1, 3, and 5 hours post-dosing for clinical signs of toxicity, then once daily for an additional 14 days. Body weights were determined prior to dosing on day 1, then on days 8 and 15 (prior to sacrifice). Food consumption was recorded daily. Following terminal sacrifice, animals underwent full necropsy and evaluation of gross pathologic

changes, including organ weights and morphologic changes. No histopathologic evaluation of tissues was performed.

There were no overt signs of clinical toxicity noted in animals from either the placebo control or the PEG-IFN treated groups. Body weight gains were not appreciably different between the two groups, and there were no changes in food consumption in the mice treated with SCH 54031 as compared to the group injected with the placebo control. At necropsy, no gross pathologic lesions were observed in any animals in either the control or treatment groups that were related to the test article. A single finding of focal hemorrhage in one lung was observed in one female mouse in the placebo control group (animal #49F) was considered incidental to treatment with the test article.

In summary, treatment of outbred, ICR mice with a single, i/v injection of SCH 54031 PEG-IFN was not associated with any overt signs of toxicity. The NOAEL for PEG-IFN in this species is > 60, 410 $\mu g/m^2$ by this route of administration.

Study #95024 (Report #P-6088). ***Acute subcutaneous toxicity study of SCH 54031 (PEG_{12000}-IFN alfa-2b) in rats.***

The acute toxicity of PEG-IFN was evaluated in Sprague-Dawley rats after a single, s/c injection of either vehicle control (SCH 54031 placebo) or 56,850 $\mu g/m^2$ SCH 54031 PEG-IFN. The rats were observed immediately after treatment and at 0.25, 0.5, 1, 3, and 5 hours post-dosing for clinical signs of toxicity, then once daily for an additional 14 days. Body weights were determined prior to dosing on day 1, then on days 8 and 15 (prior to sacrifice). Food consumption was recorded daily. Following terminal sacrifice, animals underwent full necropsy and evaluation of gross pathologic changes, including organ weights and morphologic changes. No histopathologic evaluation of tissues was performed.

There were no overt signs of clinical toxicity noted in animals from either the placebo control or the PEG-IFN treated groups. Body weight gains were not appreciably different between the two groups, and there were no changes in food consumption in the rats treated with SCH 54031 as compared to the group injected with the placebo control. At necropsy, no gross pathologic lesions were observed in any animals in either the control or treatment groups that were related to the test article. A single finding of a 1 mm^2 focal area of hemorrhage in the lung was observed in one female rat in the placebo control group (animal #9F), and was considered incidental to treatment with the test article.

In summary, treatment of outbred, Sprague-Dawley rats with a single, s/c injection of SCH 54031 PEG-IFN was not associated with any overt signs of toxicity. The NOAEL for PEG-IFN in this species is > 56,850 $\mu g/m^2$ by this route of administration.

Study #95025 (Report #P-6089). ***Acute intravenous toxicity study of SCH 54031 (PEG_{12000}-IFN alfa-2b) in rats.***

The acute toxicity of PEG-IFN was evaluated in Sprague-Dawley rats after a single, i/v injection of either vehicle control (SCH 54031 placebo) or 56,850 $\mu g/m^2$ SCH 54031 PEG-IFN. The rats were observed immediately after treatment and at 0.25, 0.5, 1, 3, and 5 hours post-dosing for

clinical signs of toxicity, then once daily for an additional 14 days. Body weights were determined prior to dosing on day 1, then on days 8 and 15 (prior to sacrifice). Food consumption was recorded daily. Following terminal sacrifice, animals underwent full necropsy and evaluation of gross pathologic changes, including organ weights and morphologic changes. No histopathologic evaluation of tissues was performed.

There were no overt signs of clinical toxicity noted in animals from either the placebo control or the PEG-IFN treated groups. Body weight gains were not appreciably different between the two groups, and there were no changes in food consumption in the rats treated with SCH 54031 as compared to the group injected with the placebo control. At necropsy, no gross pathologic lesions were observed in any animals in either the control or treatment groups that were related to the test article. Single, 1 mm^2 areas of focal hemorrhage in the lung were observed in one male and one female rat each in the group treated with SCH 54031 PEG-IFN (animals #14M and #19F, respectively), and were considered incidental to treatment with the test article.

In summary, treatment of outbred, Sprague-Dawley rats with a single, i/v injection of SCH 54031 PEG-IFN was not associated with any overt signs of toxicity. The NOAEL for PEG-IFN in this species is > 56,850 $\mu g/m^2$ by this route of administration.

Comment: Both *in vitro* and *in vivo* pharmacology studies in rats and mice have previously demonstrated that these species are not responsive to IFN. Therefore, the data from these four studies are irrelevant in demonstrating the safety of PEG-IFN for use in the clinical studies.

Study #95028 (Report #P-6135). ***Subcutaneous single dose tolerance study of SCH 54031 (PEG_{12000}-IFN alfa-2b) in cynomolgus monkeys.***

The acute toxicity of SCH 54031 PEG-IFN was evaluated in male and female cynomolgus monkeys after s/c injection. Two animals per sex received a single injection of 29435, 58861, or 117,721 $\mu g/m^2$ PEG-IFN subcutaneously into the interscapular region. Observations for overt signs of clinical toxicity were performed immediately after dosing, and at 0.25, 0.5, 1, 3, and 5 hours post-dose on day 1, then daily afterwards for 21-22 days. Changes in behavior and general condition were recorded daily. Physical examinations, including rectal body temperature, heart and respiratory rates, and blood pressure were evaluated once prior to dosing, and at 0.5, 3, 24, and 48 hours after dosing. Body weights were obtained immediately prior to dosing on day 1, then at days 8, 15, and 22 on study prior to sacrifice. Samples of peripheral blood for determination of serum IFN and anti-interferon neutralizing antibody activity were obtained from all monkeys on study, with the exception of one female monkey (animal #3F), which was sacrificed moribund on day 7. Serum interferon levels were determined by both ELISA and CPE bioassay from samples collected prior to dosing on day 1, and at 6 and 24 hours, and 7, 14, and 21 days post-dose. Samples for evaluation of anti-interferon neutralizing activity were collected prior to dosing on day 1, and at study termination on day 21, and evaluated by both enzyme immunoassay and CPE bioassay for neutralization of the anti-viral activity of IFN. Hematologic and clinical chemistry profiles were obtained only from one monkey that was sacrificed moribund at day 7 on study (animal #3F).

Only monkeys in the high-dose group were necropsied at study termination. Both female monkeys had early deaths on study (animal #3F was sacrificed moribund at day 7, while animal #4F was found dead on day 11. Complete necropsies were performed on both of these monkeys. The male

monkeys in this dose group were both euthanized, and complete necropsies performed to attempt to determine the cause of toxicity in the female animals at this dose level. The remainder of the monkeys survived the duration of the study with no overt toxicities, and were returned to the research colony at study termination.

Clinical signs included inappetence in all monkeys treated with either 58,861 or 117,721 mg/m^2 PEG-IFN, beginning days 4-5 on study. Food consumption returned to normal in all surviving monkeys by day 13. There were no effects of treatment with PEG-IFN on body weight over the duration of the study. On evaluation of physiologic parameters, there were no effects of PEG-IFN treatment on respiratory rates or heart rates in all groups of treated monkeys. At 48 hours after injection, individual body temperatures ranged from 1.8 to 3.5°F lower than baseline in all 4 monkeys treated with 117,721 $\mu g/m^2$ PEG-IFN, and in 1 male monkey and both female monkeys treated with 58,861 $\mu g/m^2$ SCH 54031. Mean arterial blood pressure, as well as individual systolic and diastolic blood pressures were also decreased in all 4 monkeys treated with the high-dose PEG-IFN at 24 hours post-dosing, which was possibly related to treatment with the test article.

Comment: Mild hypotension has previously been reported in patients treated with unconjugated IFN, and is usually asymptomatic.

In summary, a single, s/c dose of 58860 or 117721 $\mu g/m^2$ PEG-IFN in cynomolgus monkeys was associated with transient inappetence, hypothermia, hypotension, and death in the two females treated at the highest dose. The NOAEL, based on clinical observations, was 58860 $\mu g/m^2$ for male monkeys, and 29440 $\mu g/m^2$ in female animals. These doses represent approximately 1500 and 750 times the dose of 1 μg/kg used in the pivotal clinical trial.

Study #94081 (Report #P-6136). ***One-month subcutaneous toxicity study of SCH 54031 (PEG_{12000}-IFN-α 2b) solution in cynomolgus monkeys.***

The toxicity and toxicokinetics of SCH 54031 PEG-IFN after daily, s/c injections were evaluated in cynomolgus monkeys after a one month treatment period. Male and female cynomolgus monkeys (3/sex/group) were treated every other day with vehicle control (PEG-IFN placebo), INTRON®-A (3105 $\mu g/m^2$), or PEG-IFN at doses of 1414, 4239, or 14126 $\mu g/m^2$/injection. Two additional animals/sex in the control and high-dose PEG-IFN groups were retained for a 4 week treatment-free recovery period. Clinical observations for signs of morbidity or overt toxicities, as well as measurement of food consumption were performed daily, and body weights were determined weekly. Fasting peripheral blood samples for hematologic and serum biochemistry profiles were obtained twice during the pre-test period for determination of baseline values, then after two and four weeks of treatment with PEG-IFN or controls, and at week 4 of recovery in the appropriate dose groups. Urinalysis and urine chemistries, physiologic parameters (ECG, respiratory and heart rates, blood pressure, body temperatures) were recorded at these same time points. General veterinary examinations, as well as ophthalmologic examinations were performed once during the baseline period, then at week 4 on study and at week 8 in the recovery animals. A full necropsy and gross pathologic evaluation was performed on each animal at terminal sacrifice (weeks 5 or 9 on study for the end-of-treatment and recovery groups, respectively), with organ weights recorded, and tissue samples taken and processed for histopathologic evaluation.

Peripheral blood samples were also obtained from SCH 54031 and INTRON®-A-treated monkeys for companion toxicokinetic and antibody development assays (please see Study #P-6151, above). Serum samples were collected on days 1 and 5 of study prior to treatment, then at 4, 8, 12, 24, 36, and 48 h after the first dose of PEG-IFN or INTRON®-A, and on day 5 at 0, 4, and 48 h post-dose. Serum was also collected for toxicokinetic determination of PEG-IFN levels 24 h following the final dose of SCH 54031, and at terminal sacrifice following the 4-week recovery period. Serum interferon levels were measured both by ELISA, and by specific inhibition of viral cytopathic effect (CPE bioassay). Antibody activity (both total Ig and neutralizing antibody) were determined by enzyme immunoassay (EIA) and the CPE bioassay, respectively.

One female monkey (#35F) was sacrificed moribund on d 22, after receiving approximately 9 doses of 14,130 $\mu g/m^2$ SCH 54031, s/c. During the second week on study, this animal exhibited signs of inappetence and decreased food consumption, resulting in scant feces and a 0.5 kg loss of body weight from baseline by week 3 on study. Other clinical signs included weakness and hypoactivity, emaciation, coolness to the touch, tremors and dehydration, beginning during the third week of treatment. Monkey #35F also developed several abscesses at the injection sites for PEG-IFN, which on histologic evaluation were associated with moderate to severe, suppurative cellulitis and presence of bacteria in the lesions.

No hematology parameters could be evaluated for this animal at sacrifice, as the sample was clotted; however, red cell counts and hemoglobin levels in this monkey were decreased from the two baseline measurements at the 2 week time point, suggesting a slight anemic effect. The decreases in erythrocyte parameters were also observed in 3/ 4 of the other female monkeys, as well as in all male monkeys in this dose group at weeks 2 and 4 on study, as compared to baseline. Total leukocyte counts for this animal decreased by almost 75% as compared to baseline for monkey #35F at week 2 on study, with a value of 5.3 x $10^3/mm^2$ at this time point, as compared to 20.1 and 13.6 x $10h^3/mm^2$ at weeks –3 and –1, respectively, prior to treatment. Similar 70-80% decreases in both neutrophil and lymphocyte numbers at the week 2 time point were also observed in this animal at the week 2 time point.

The serum biochemistry samples taken from monkey #35F at terminal sacrifice revealed two-fold elevations of both ALT and AST, as compared to the weeks –3 and –1 baseline values, and to week 2 on study. Decrease in serum glucose and creatinine to 46 g/dL and 0.6 mg/dL, respectively, and 30-50% decreases in total protein, albumin, and globulin levels as compared to baseline were also observed in this monkey at the terminal sacrifice. No other remarkable changes in serum biochemistry parameters were observed for this animal.

Comment: The values for the hematology profiles in this animal at terminal sacrifice could not be determined, due to clotting of the samples. The values for the clinical biochemistry profiles do not identify whether these samples were also clotted and/or hemolyzed, but in some cases (*e.g.* the potassium and glucose values) the week 4 values are marked "Physical characteristics may have interfered with the assay." From the data contained in the final toxicology report, it is not possible to determine whether some of the findings (*i.e.* the elevated ALT and AST values) were due to problems in sample collection or were true effects of the PEG-IFN treatment.

Gross pathologic findings at necropsy of monkey #35F included minimal to mild effusions in the abdominal cavity and pericardium, respectively, which had no correlating pathology on histologic evaluation, as well as the abscesses described above. No pathologic changes were evident on gross evaluation in the spleen, thymus, and bone marrow; however, microscopic evaluation revealed

moderate hypocellularity in the bone marrow and cortical atrophy in the thymus, with corresponding lymphocyte depletion in the spleen. Similar lymphocyte depletion and evidence of atrophy were evident both on gross and microscopic pathologic examination of the mesenteric lymph nodes, in addition to sinusoidal erythrocytes and erythrophagocytosis. These changes were considered by the sponsor to be an extension of the marrow suppressive effects of interferon-α, resulting in anemia, decreased total protein synthesis by the liver, and decreased leukocyte responses to infection.

During the treatment period, the remainder of the animals treated with the highest dose of SCH 54031 showed clinical signs of toxicity. Inappetence, scant feces, and poor food consumption were noted in beginning at week 2 and increasing in severity at week 3 in 4/5 female monkeys in the highest dose group (animals #35F, #36F, #37F, and #38F), and in one male (animal #32M) at 2 weeks and one male (animal #33M) at 3 weeks on study. Three of the female monkeys (animals #35F, #36F, and #38F) were also dehydrated and cool to the touch, while animal #38F also developed diarrhea. However, beginning in week 3, normal food consumption was resumed in all of these animals except #35F (see above), and by study termination, all of the male animals, and 2/5 of the female monkeys in this dose group had returned to baseline weight values. Animals held for the 4-week recovery period continued to gain weight over the duration of the study, suggesting that the inappetence caused by PEG-IFN treatment was reversible following cessation of dosing.

Monkeys in the mid-dose group also displayed overt signs of toxicity; however, the severity of the effects was generally less than those observed in the high-dose group. All monkeys treated with 4239 $\mu g/m^2$ PEG-IFN survived for the duration of the study, and inappetence and/or weight loss were not observed in any of the male animals in this dose group. The female monkeys had slight (0.1 –0.2 kg) losses in body weight, however the severity was less than 5 to 10% decrease from initial body weight. Scant feces were noted on two occasions in two female monkeys in this dose group (animals #27F and #28F) at week 2 on study, and animal #27F also had 2 episodes of diarrhea during the same time period. During week 3 on study, animal #28F was noted to have scant feces on one occasion, as were monkeys #24M (male) and #26F (female) in the mid-dose group. One male monkey in this dose group (animal #25M) also exhibited a 3 x 3 cm area of swelling in the inter-scapular region of the beck (near the injection sites) on two occasions during week 2 and on 3 occasions during week 3; however, this finding had completely resolved by the time of terminal sacrifice at the end of week 4.

There were no overt clinical signs of toxicity seen in any of the placebo control group animals, nor in the animals treated with 3105 $\mu g/m^2$ INTRON®-A as a positive control, with the exception of two episodes of red-streaked diarrhea in one male monkey (#15M) during week 2. A single occasion of loose stool was noted in one female monkey (#20F) treated with the lowest dose of 1414 $\mu g/m^2$ PEG-IFN during the second week of treatment. By week 4 on study, there were no observable clinical abnormalities in any of the monkeys in all dose groups.

There were no significant changes in rectal body temperature, heart rate, blood pressure, respiratory rates, or ECG profiles in monkeys treated for two or four weeks with either SCH 54031 PEG-IFN or INTRON®-A, as compared to the placebo control group or to baseline values. Ophthalmologic exams were normal in all monkeys at all time points on study, and there were no remarkable findings on urinalysis profiles.

Hematologic profiles showed evidence of anemia, decreased total leukocyte counts, and decreased platelets in all groups of monkeys treated with either INTRON®-A or SCH 54031, beginning at the week 2 time point. Dose-related, 5 to 20% decreases in mean red cell counts, hematocrit, and hemoglobin concentration were observed in both male and female monkeys treated with PEG-IFN, as compared to either baseline values, or to the placebo control group. These changes persisted at the 4 week time point, and were only partially resolved after a 4-week treatment-free recovery period, suggesting that the marrow suppressive effects of the pegylated interferon-α are related to its extended half-life, as compared to INTRON®-A.

At week 2 on study, mean platelet counts were also decreased from baseline by 26 to 52% in all groups of monkeys treated with PEG-IFN, and by approximately 33% in monkeys treated with INTRON®-A. In contrast to the erythrocyte parameters, however, platelet counts had recovered to baseline values in almost all groups at week 4 on study, and continued to increase over baseline during the treatment-free recovery phase. At week 2, there was a trend towards slight elevations in prothrombin time and activated partial thromboplastin (APTT) times in the groups of animals treated with SCH 54031 as compared to the placebo controls. Statistical analysis (one-way ANOVA) revealed that while there were no statistically significant differences in prothrombin times between the placebo and interferon-treated groups, dose-related increases in APTT at the 2 week time point were statistically different ($p \leq 0.01$, ANOVA) from the placebo control group in both the male and female monkeys treated with PEG-IFN. By the end of the 4 week treatment period, APTT and prothrombin times were not statistically different from either control animals or from baseline values in the any of the groups of PEG-IFN or INTRON®-A treated monkeys, and all values had returned to baseline by the end of the 4-week recovery period.

Total leukocyte counts were initially decreased by greater than 50% as compared to either baseline values or the placebo control in all groups of interferon-treated monkeys after two weeks on study ($p \leq 0.01$, ANOVA), with no apparent relationship in either the incidence or severity of the decreases to the dose of PEG-IFN. Recovery of total leukocyte counts was observed in all groups of animals at the week 4 time point and at the end of the recovery period; although not all mean values had reached baseline by that time, there were no statistically significant differences between the groups. Of interest, there was an increase in the mean white cell counts in the mid-dose group at 4 weeks on study, suggesting an apparent rebound effect from the initial decrease at week 2. These data are presented in Table VI, below.

Table VI – Total Peripheral Blood Leukocyte Counts Following IFN Treatment

Dose of PEG-IFN	Mean Total Leukocyte Counts (x 1000/mm^3) ± S.D.			
	Baseline	Week 2	Week 4	Recovery
Placebo	14.4 ± 2.7	10.9 ± 2.5	10.9 ± 1.5	10.5 ± 2.3
INTRON®-A	11.7 ± 3.4	4.8 ± 1.1[a,b]	9.9 ± 1.7	n.d.[c]
1413 μg/m^2	12.0 ± 2.5	5.1 ± 1.2[a,b]	11.5 ± 1.7	n.d.[c]
4239 μg/m^2	12.8 ± 3.0	5.4 ± 1.3[a,b]	16.0 ± 4.1[a]	n.d.[c]
14129 μg/m^2	13.3 ± 3.0	4.7 ± 1.3[a,b]	13.6 ± 10.8	10.7 ± 4.6

[a] significantly different from placebo control ($p \leq 0.01$, ANOVA)
[b] significantly different from baseline ($p \leq 0.001$, ANOVA)
[c] n.d. = not done

Similar 30 to >75% decreases in both absolute neutrophil counts and lymphocyte counts were observed in interferon-treated monkeys at the week 2 time point, as compared to either placebo control or to baseline values. Once again, there was no relationship to either the severity or the incidence of the changes to the dose of SCH 54031 administered to the monkeys. One male monkey (#33M) in the group of animals treated with 14129 $\mu g/m^2$ PEG-IFN had a significantly elevated neutrophil count ($p \leq 0.001$, Student's *t* test) at the end of the 4-week treatment period as compared to the mean baseline value for the group; however, this animal was maintained for recovery and the neutrophil count had returned to less than baseline at the end of the study. Recovery of both neutrophil and total lymphocyte counts to approximately baseline values was observed in all groups of animals treated with either PEG-IFN or INTRON®-A by the end of week 4 on study. These data are presented in Table VII and VIII, below.

Table VII – Peripheral Blood Neutrophil Counts Following IFN Treatment

Dose of PEG-IFN	Mean Neutrophil Counts (x 1000/mm^3) ± S.D.			
	Baseline	Week 2	Week 4	Recovery
Placebo	7.5 ± 3.3	5.7 ± 2.8	5.3 ± 1.8	3.4 ± 1.2
INTRON®-A	5.5 ± 2.4	1.0 ± 0.3[a,b]	5.2 ± 2.7	n.d.[c]
1413 $\mu g/m^2$	6.5 ± 2.2	1.5 ± 1.0[a,b]	5.7 ± 2.7	n.d.[c]
4239 $\mu g/m^2$	6.1 ± 0.5	1.3 ± 1.2[a,b]	10.1 ± 3.9[a]	n.d.[c]
14129 $\mu g/m^2$	6.5 ± 2.4	2.0 ± 1.1[a,b]	7.4 ± 9.8	4.2 ± 3.3

[a] significantly different from placebo control ($p \leq 0.01$, ANOVA)
[b] significantly different from baseline ($p \leq 0.001$, ANOVA)
[c] n.d. = not done

Table VIII – Peripheral Blood Lymphocyte Counts Following IFN Treatment

Dose of PEG-IFN	Mean Lymphocyte Counts (x 1000/mm^3) ± S.D.			
	Baseline	Week 2	Week 4	Recovery
Placebo	6.0 ± 1.4	4.5 ± 1.6	5.1 ± 1.9	6.2 ± 1.4
INTRON®-A	5.4 ± 0.9	3.2 ± 0.9[a]	4.1 ± 1.0	n.d.[c]
1413 $\mu g/m^2$	4.7 ± 1.1	3.1 ± 1.0[a]	5.0 ± 1.8	n.d.[c]
4239 $\mu g/m^2$	5.8 ± 2.4	3.6 ± 1.1[a]	4.9 ± 2.1	n.d.[c]
14129 $\mu g/m^2$	6.0 ± 1.7	2.3 ± 0.8[a,b]	5.0 ± 1.7	5.8 ± 2.2

[a] significantly different from baseline ($p \leq 0.05$, ANOVA)
[b] significantly different from placebo control ($p \leq 0.01$, ANOVA)
[c] n.d. = not done

There were no remarkable changes in eosinophil, basophil, atypical lymphocytes, or immature (band) neutrophils in any of the interferon-treated monkeys, as compared to either the placebo control group or to baseline values. At week 4, monocyte counts were elevated by approximately 2-fold as compared to baseline in one male and one female monkey (#33M and #34F, respectively) in the high-dose PEG-IFN group, however, the monocyte counts for these two animals returned to less than the initial values by the end of the 4-week recovery period. There were no other remarkable changes in monocyte counts in any of the other dose groups.

There were no definitive, treatment-related changes in clinical chemistry profiles for animals treated with 1414, 4239, or 14132 μg/m^2 SCH 54031 at either 2 or 4 weeks, as compared to either the placebo control group, or to baseline values. Slight, although statistically not significant elevations in total bilirubin, as compared to either baseline values or to the placebo control group were noted at week 2 on study in the high-dose PEG-IFN treated group; however, these findings had resolved to baseline by the end of the 4-week treatment period. Decreases in total protein, globulin, and serum albumin levels, with concomitant 25 to 305 decreases in A:G ratios were noted in all groups of interferon-treated monkeys without an apparent relationship to the dose of PEG-IFN, beginning at week 2 and continuing until study termination. These changes had only partially resolved at the end of the 4-week recovery period in the high-dose male and female, PEG-IFN treated monkeys.

At necropsy, the only treatment-related macroscopic pathology finding was discoloration at the injection site (redness, bruising). This effect was observed in monkeys in all groups, including both the vehicle and INTRON®-A controls without a dose-relationship in either incidence or severity. Other, incidental findings included parasitic nodules in the colon, cecum, and jejunum of several animals, small testes in one male monkey each in the control, INTRON®-A, and mid-dose PEG-IFN treated groups (suggesting a sexually immature animal), and adhesions and areas of discoloration and consolidation in the lungs from two female monkeys, in the low and mid-dose PEG-IFN groups, respectively.

Histologic findings related to treatment were limited to the injection site, and included subcutaneous hemorrhage, inflammation, perivasculitis and endarteritis, and fibrosis. These findings occurred with approximately equal incidence and severity in all of the treatment groups, including the controls. The two females showing macroscopic changes in the lungs had findings on histologic evaluation which correlated with the gross pathology, including an area of infarct in the low-dose female monkey and areas of pneumonitis and pneumoconiosis in the lungs from the mod-dose female animal. Other microscopic findings included increased cellularity in the bone marrow in animals of both sexes, in all treatment groups including the controls, mononuclear cell infiltrates, mineral deposition, and tubular pigmentation in the kidneys, increased pigment and lymphoid hyperplasia in the mesenteric and salivary lymph nodes, and focal areas of hepatocellular vacuolization, necrosis, and mononuclear cell infiltrates in the liver. These findings occurred at approximately equal incidence and severity across all treatment groups, and were considered by the reviewing pathologist to be incidental to treatment with the test articles.

Comment: Serum interferon and neutralizing antibody levels in this study were previously reviewed under pharmacokinetics study #P-6135, and will not be reported here. However, it should be noted that the reversal of the hematologic toxicities observed in the present study was correlated with the development of neutralizing antibody titers to interferon.

In summary, treatment of both male and female cynomolgus monkeys with PEG-IFN for one month was associated with inappetence in the absence of significant weight loss, decreases in erythrocyte and platelet counts, and both total leukocyte and absolute neutrophil numbers. Pathologic findings related to PEG-IFN treatment included macroscopic and microscopic evidence of hemorrhage, inflammation, and fibrosis at the injection site. These findings were transient in nature, and had resolved or were in the process of resolving by the end of the 4 week recovery period. Reversal of toxicity was associated with development of neutralizing antibodies in all of the groups treated with PEG-IFN, and in the animals treated with 3105 μg/m^2/dose INTRON®-A

as a positive control. The NOAEL for PEG-IFN administered every other day by s/c injection for one month to cynomolgus monkeys was 1414 μg/m^2/dose, based on the hematologic findings.

Study #95029 (Report #P-6102). ***Pain on injection study of a parenteral formulation of SCH 54031 (PEG_{12000}-IFN-α 2b) in rats.***

The potential for PEG-IFN to induce pain on injection was evaluated in female, Sprague-Dawley rats. Ten rats per group received a single, sub-plantar injection of 35 μg PEG-IFN, SCH 54031 placebo, or INTRON®-A. Control animals were injected with 0.1 ml 0.9% sterile saline or Mefoxin® (400 mg/ml) as the negative or positive controls, respectively. Each rat was observed for 12 minutes for signs of pain after injection (as evidenced by paw lifting or licking), that were graded on a scale of 0 to 3. A pain rating was calculated for each rat for every 3 minute interval using previously described methods[3], and the results are presented in the table, below:

Table IX – Pain on Injection of PEG-IFN in Female Rats

	Mean Pain Rating, ± S.D.			
	Time after Dosing (minutes)			
Group	0-3	3-6	6-9	9-12
0.9% saline	0 ± 0	0.03 ± 0.11	0 ± 0	0 ± 0
SCH 54031 placebo	0.01 ± 0.03	0.08 ± 0.16	0.01 ± 0.02	0.01 ±0.02
SCH 54031, 35 μg	0 ± 0	0.03 ± 0.08	0.01 ± 0.03	0 ± 0
INTRON®-A	0.03 ± 0.05[a]	0.03 ± 0.1	0.01 ± 0.02	0 ± 0
Mefoxin®	2.12 ± 0.2[b]	2.40 ± 0.43[b]	2.18 ± 0.15[b]	1.79 ± 0.38[b]

[a] significantly different ($p \leq 0.05$) from SCH 54031 (Mann-Whitney U-test)
[b] significantly different ($p \leq 0.001$, Mann-Whitney U-test) from SCH 54031, SCH 54031 placebo, INTRON®-A, and saline control

Injection of Mefoxin® caused the expected pain response in rats, both in severity and duration, in all ten rats in this dose group. Transient pain was observed in 4/10 animals injected with INTRON®-A at the earliest time point and 1/10 rats 3-6 minutes after injection. By contrast, the pain on injection ratings of either SCH 54031 PEG-IFN or SCH 54031 placebo were not statistically different from saline control at any time point tested.

In summary, under the conditions of the present study, PEG-IFN or SCH 54031 placebo showed little or no potential to cause pain on injection, as compared to the antibiotic Mefoxin®, which has known irritating properties. The reaction to PEG-IFN was not appreciably different from that observed following injection of sterile saline.

[3] Dubuisson, D. and S.G. Dennis. 1977. The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation on rats and cats. *Pain*, **4**:161-174.

Study #95030 (Report #P-6082). ***Acute subcutaneous irritation study of a new parenteral formulation of SCH 54031 (PEG_{12000}IFN alfa-2b) in rats.***

The local irritation effects of SCH 54031 PEG-IFN were evaluated in male Sprague-Dawley rats after a single, s/c injection. Five animals per group, per time point were dosed with 105 µg PEG-IFN, 0.3 ml SCH 54031 placebo, or 57.6 µg INTRON®-A. Control animals were injected with 0.3 ml 0.9% sterile saline or Mefoxin® (400 mg/ml) as the negative or positive controls, respectively. Macroscopic observations for local irritation were performed daily, but were not graded for extent or severity of effect. Rats were euthanized following the final observations 24, 48, and 96 hours after injection, and representative tissue samples from each injection site and the surrounding tissues evaluated histologically for inflammation and irritation. Grading of local irritation was done on a scale of 0 to 5 for intensity of effect, and on a scale of 0 to 3 for extent, according to the methods described by Vranckx[4]. Both scores were considered in the evaluation of the irritation potential of the test article.

No visible evidence of pain or discomfort, swelling, erythema, or heat at the injection site was noted in any of the rats following injection with the saline or placebo controls, INTRON®-A, or PEG-IFN, at any time point on study. One rat (animal #71M) in the group injected with Mefoxin® as a positive control developed swelling at the injection site one hour post-dose, and scabs over the injection site developed in this animal and rat #70M beginning 24 hours after dosing and continuing until terminal sacrifice at 96 hours after treatment.

Histologically, there was no evidence of inflammation or irritation 24 hours after treatment in the rats injected with PEG-IFN, while all Mefoxin®-dosed rats had local inflammatory responses of Grade 2-3 in both intensity and extent of tissue involvement. Grade 1-3 inflammatory responses were observed in 1/5 saline-injected rats, and in 3/5 rats each in the groups treated with either SCH 54031 placebo or INTRON®-A at the 24 h time point as well.

At 48 hours, one rat each in the SCH 54031 placebo-injected group had Grade 1 or 2 irritation at the injection site, while no evidence of inflammation or irritation was present in any of the rats injected with the saline control vehicle. Irritation was present (Grade 2) in 1/5 rats treated with PEG-IFN, and in 3/5 rats treated with Mefoxin®. One rat injected with INTRON®-A showed Grade 3 intensity and extent of irritation 48 hours after treatment.

At 96 hours after injection, the tissue irritation and inflammation in the saline, SHC 54031 placebo, SCH 54031 PEG-IFN, and INTRON®-A treated groups had resolved. Local tissue irritation accompanied by minimal to severe hemorrhage, edema, mononuclear cell infiltrates, fibrinous exudates, vascular thromboses, fibroplasia and immature granulation tissue, and inflammation and necrosis of the underlying muscle was present in all of the Mefoxin®-injected rats.

In summary, s/c injection of PEG-IFN in male Sprague-Dawley rats was associated with transient, minimal to mild evidence of local irritation, which was not appreciably different from the findings in animals injected with either saline or SCH 54031 placebo as controls. By contrast, injection of

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[4] Vranckx, C.H. 1968. A simple method for quick evaluation of local tolerance to injected drugs. *In*: Proceedings of the European Society of Drug Toxicology, Volume IX; Excerpta Medica Formulation, New York, New York, pp. 308-310.

the antibiotic Mefoxin® led to prolonged irritation and inflammatory changes, as well as early development of tissue necrosis and granulation responses at the injection site.

Study #95031 (Report #P-6083). ***Muscle irritation study of a parenteral formulation of SCH 54031 (PEG_{12000}IFN alfa-2b) in rabbits.***

The local irritation effects of SCH 54031 PEG-IFN were evaluated in New Zealand white rabbits after a single, i/m injection. Four animals per group, per time point were dosed with 350 µg PEG-IFN, SCH 54031 placebo, or 192 µg INTRON®-A in a volume of 1.0 ml. Control animals were injected with 1.0 ml 0.9% sterile saline or Mefoxin® (400 mg/ml) as the negative or positive controls, respectively. Macroscopic observations for local irritation were performed daily, and any findings were rated numerically on a scale of 0 to 5 for evidence of local irritation, erythema, and/or edema by the methods of Shintani *et al*[5]. Rabbits were euthanized following the final observations 24, 72, and 168 hours after injection, and representative tissue samples from each injection site and the surrounding tissues evaluated histologically for inflammation and irritation. Grading of local irritation was done on a scale of 0 to 4 for severity, according to the methods of Draize[6].

One rabbit in the group treated with PEG-IFN (animal #14) was found dead on day 4 after injection. Gross evaluation on necropsy revealed evidence of a distended cecum filled with mucoid contents and red discoloration of the cecal walls and the lungs. The cause of death in this animal was determined to be mucoid enteritis, and was unrelated to treatment with PEG-IFN.

Gross evidence of muscle irritation was present in all groups of treated rabbits, including the saline and placebo controls. Mean lesion sizes in the group of rabbits injected with SCH 54031 PEG-IFN were 1564, 1096, and 152 mm^3 at 24, 72, and 168 hours after injection, respectively. Mean irritation scores increased from 2.25 at 24 hours to 2.75 at 72 hours after injection, then decreased to 1.67 by study termination at 168 hours post-dose. Histologic changes were minimal to mile (Grade 1-2) in severity at all time points after injection. At 24 hours after dosing, findings included focal areas of muscle cell degeneration and necrosis, with occasional areas of hemorrhage and/or mononuclear cell infiltrations. The severity of necrosis and leukocytic infiltrates was increased slightly to Grade 2 at 72 hours after injection, and had resolved by 168 hours post-dose, with evidence off muscle cell regeneration, and mild atrophy and fibroplasia present.

Mean irritation scores in rabbits injected with 0.9% saline were minimal at all time points on study, for both gross and microscopic evaluation. Macroscopic evaluation of muscle irritation in SCH 54031 placebo-treated animals showed a similar pattern to that observed for animals injected with PEG-IFN, although the both the size and severity of the lesions was decreased compared to the findings with the test article. Mean irritation scores at 24, 72, and 96 hours in this group of rabbits were 1.25, 1.75, and 0.75, respectively, and mean lesion size was 600, 675, and 120 mm^3, respectively, at these same time points.

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[5] Shintani, . 1967. A new method to determine the irritation of drugs after intramuscular injection in rabbits. *Toxicol. Appl. Pharmacol.*, **11**:293-301.

[6] Maurer, T., P. Thomann, E.G. Weirich, and R. Hess. 1978. Predictive evaluation in animals of the contact allergenic potential of medically important substances. I. Comparison of different methods of inducing and measuring cutaneous sensitization. *Contact Dermatitis*, **4**:321-333.

INTRON®-A irritation scores and lesion sizes decreased at each successive time point, with mean irritation scores of 1.75 at 24 hours and 0.33 at 168 hours after injection, and respective mean lesion sizes of 1483 and 3 mm^3. By contrast, injection of Mefoxin® produced the expected, substantial local irritation, with irritation scores for individual animals between 4.5 and 5 at all time points. Lesion sizes after Mefoxin® ranged from 1404 to 7500 mm^3 at 24 hours post injection, and decreased slightly to 1584 to 6237 mm^3 by 72 to 168 hours post-treatment. Histologically, severe, coagulative necrosis of muscle fibers (Grade 4) and inflammatory cell infiltrates were present 24 and 72 hours after injection of Mefoxin®, followed by muscle cell atrophy, regeneration, fibroplasia, and prominent cellular infiltrates at the 168 hour time point.

In summary, s/c injection of PEG-IFN in male New Zealand white rabbits was associated with transient, minimal to mild evidence of local irritation, which was not appreciably different from the findings in animals injected with either saline or SCH 54031 placebo as controls. By contrast, injection of the antibiotic Mefoxin® led to prolonged irritation and inflammatory changes, as well as early development of tissue necrosis and granulation responses at the injection site.

Study Report : ——— . ***Acute toxicity study of Sch 30500 (alpha-2 INTERFERON) in Rhesus monkeys.***

Comment: This study was previously submitted and reviewed under PLA #83-415. A brief summary of the results is provided here, for reference purposes only.

The acute toxicity of IFN was evaluated in Rhesus macaques after a single i/v or i/m injection. Four monkeys per sex were injected with either vehicle (diluent for IFN, agent not specified), 130, or 260 x 10^6 IU IFN/kg i/m, or 260 X 10^6 IU IFN/kg, i/v. There were no clinical toxicities, alterations in general condition, body weight, or food consumption over the 14 d duration of the study. No remarkable increases in rectal body temperature were noted during the first eight hours after dosing, and there were no significant changes in total leukocyte counts, hematologic, or serum biochemistry profiles either 24 hours or 14 days after dosing with the test article, as compared to the control group. Analysis of serum samples taken at various time points after treatment revealed maximal IFN levels of approximately 9 x 10^5 IU/ml (by CPE assay) in the group treated with 260 x 10^6 IU/kg IFN by the i/v route at 30 min after injection, and between $10^{4.34}$ and $10^{5.14}$ IU/ml 1-2 hours after i/m dosing. Serum interferon levels remained elevated between $10^{3.66}$ to $10^{4.03}$ IU/ml (by CPE assay) in all groups of monkeys treated with IFN 24 hours after dosing, and had decreased to $10^{1.48}$ to $10^{2.27}$ IU/ml by day 14.

In summary, a single administration of 130 or 260 x 10^6 IU/kg of unconjugated IFN by either i/m or i/v injection in Rhesus monkeys resulted in no remarkable clinical, hematologic, or biochemical toxicities at serum IFN levels similar to those observed with low doses of PEG-IFN.

Study #80094 (Report #P-4782). ***One-month intramuscular toxicity study of SCH 30500 (α2-interferon) in the rat.***

Comment: This study was previously submitted and reviewed under PLA #83-415. A brief summary of the results is provided here, for reference purposes only.

The toxicity of repeat, daily administrations of IFN was evaluated in Sprague-Dawley rats. Fifteen rats per sex were injected i/m with either vehicle control (sterile, phosphate buffered saline) or 1.1 MIU/kg IFN daily for four weeks, and all animals were sacrificed at the end of the treatment period. There were no overt signs of toxicity, including no aberrant clinical observations, no changes in body temperature after IFN administration, not effects on hematologic, serum biochemistry, or urinalysis profiles, and no adverse ophthalmologic findings during the duration of the study. No remarkable macroscopic or microscopic evidence of organ pathology was present in the IFN-treated rats, as compared to the vehicle controls at terminal sacrifice. Peripheral blood samples were collected for evaluation of serum IFN levels by CPE bioassay at days 1 and 22 on study, and showed increasing levels over time suggestive of bioaccumulation of the protein. Mean serum IFN levels in control rats at one hour after injection on days 1 and 22 were 39 $\pm$ 4 IU/ml and 34 $\pm$ 3 IU, respectively. By contrast, mean serum IFN levels in the treated rats were 567 $\pm$ 56 IU/ml on day 1, and 2326 $\pm$ 406 IU/ml on day 22 at one hour post-dosing. Levels of IFN in 24 hour urine specimens from the two groups ranged between 30 and 40 IU/ml at both the day 1 and day 22 time point, and were not appreciably different between the control and IFN-treated rats.

In summary, daily i/m injection of SCH 30500 (INTRON®-A) for one month in CD® rats was not associated with any clinical, macroscopic, or microscopic evidence of toxicity. The NOAEL for IFN in this species is 1.1 x 10^6 IU/kg/day.

Comment: IFN is pharmacologically active only in humans and non-human primates, and not in rodent species.

Study #80093 (Report #P-4783).). ***One-month intramuscular toxicity study of SCH 30500 (α2-interferon) in the monkey.***

Comment: This study was previously submitted and reviewed under PLA #83-415. A brief summary of the results is provided here, for reference purposes only.

The toxicity of repeat, i/m administration of IFN in cynomolgus monkeys was determined in a one month toxicity study. Three monkeys per sex were injected i/m with either vehicle control (sterile, phosphate buffered saline) or 1.1 MIU/kg IFN daily for four weeks (28-29 days). During the course of the treatment, there were no overt signs of toxicity, including no aberrant clinical observations, no changes in body temperature after IFN administration, nor effects on hematologic, serum biochemistry, or urinalysis profiles, and no adverse ophthalmologic findings observed in either the control or the IFN-treated animals. Compared to baseline, final body weights were decreased between 0.2 to 0.4 kg in 5/6 control monkeys and 6/6 IFN-treated animals, although no remarkable decreases in food consumption were noted. At necropsy, there was no treatment-related evidence of organ pathology present in the IFN-treated monkeys, as compared to the vehicle control animals. Peripheral blood samples were collected for evaluation of serum IFN levels by CPE bioassay at days 1 and 22 on study, and showed increasing levels over time suggestive of bioaccumulation of the protein. Mean serum IFN levels in control monkeys at one hour after

injection on days 1 and 22 were 46 IU/ml and 30 IU/ml, respectively, while mean serum in the treated monkeys were 613 IU/ml on day 1, and 1288 IU/ml on day 22. Levels of IFN in 24 hour urine specimens from the two groups ranged between 30 and 50 IU/ml at both the day 1 and day 22 time point, and were not appreciably different between the control and IFN-treated monkeys.

In summary, daily i/m injection of SCH 30500 (INTRON®-A) for one month in cynomolgus monkeys was not associated with any clinical, macroscopic, or microscopic evidence of toxicity. The NOAEL for IFN in this species, by this route of administration is 1.1 x 10^6 IU/kg/day.

Study #84061 (Report #P-5086). ***Three-month intramuscular toxicity study of SCH 30500 (a-2 interferon) in monkeys.***

Comment: This study was previously submitted and reviewed under PLA #89-0381. A brief summary of the results is provided here, for reference purposes only.

The potential for cumulative toxicity of SCH 30500 (INTRON®-A, IFN) was determined in a three month, repeat dose toxicology study in juvenile cynomolgus monkeys, administered daily, i/m injections of the test article. Four animals/sex/group were treated daily for 91-92 days with vehicle control (sterile, 0.9% saline), or 4, 20, or 100 x 10^6 IU/kg/day IFN, i/m. Monkeys were observed at least once daily for clinical signs of toxicity, and complete physical evaluations, including rectal body temperature, respiratory and heart rates, blood pressure, and electrocardiograms were performed at weeks –7 and –2 prior to treatment, and at weeks 4 and 13 on study. Peripheral blood samples for evaluation of serum biochemistry and hematology profiles were obtained at weeks –7 and –2 prior to treatment, and at weeks 2, 4, and 13 on study. Additional blood samples for determination of serum neutralizing antibody activity were obtained at baseline and weeks 4 and 13 on study, and at week 2 from animals in the control and high-dose groups only. Animals were sacrificed at the end of dosing, and complete necropsies, including both gross and microscopic evaluations of organ pathology were performed.

Two female monkeys (animals #31F and #29F) in the group treated with 100 MIU/kg/d IFN were sacrificed moribund at week 2 during treatment. Clinical signs of toxicity included dehydration, hyperemic oral mucosae, hypothermia, hypoactivity, and decreases in heart and respiration rates and blood pressure. Additionally, monkey #29F had periorbital edema and sloughing of the skin around the eyes, gingival bleeding, ataxia, and pale oral mucosae prior to sacrifice. All other animals survived until study termination; however, hyperemic oral mucosae with occasional gingival bleeding and dehydration were noted in all surviving high-dose animals, during weeks 2 to 3 on study. Two of eight monkeys in the group treated with 20 MIU/kg/d IFN also exhibited hyperemic oral mucosae in this same time period.

Decreased body weights and food consumption were noted in the two female monkeys (#29F and #31F) in the group treated with 100 MIU/kg/d IFN prior to sacrifice. The remainder of the animals either maintained their initial body weights, or gained weight (range 0.1-0.8 kg) over the duration of the study. Food consumption in the surviving animals was comparable between the control and IFN-treated monkeys.

In one female animal each in the high and mid-dose groups (animals #32F and #24F, respectively), the first menses during week 1 of dosing was prolonged to 9-10 days in duration, as compared to

the normal value of 5 days for this strain of monkey. All subsequent menses were of normal duration in all female monkeys on study.

In all other surviving animals, there were no remarkable effects of IFN treatment on body temperature, heart or respiratory rates, blood pressure, or ECG profiles during the 13 week treatment period. No changes in ophthalmologic parameters or urinalysis profiles were observed that were related to IFN treatment.

Transient decreases in erythrocyte parameters (*i.e.* red cell counts, hemoglobin, and hematocrit), platelet counts, and increases in APTT (animal #28 only) were observed 3 to 4 monkeys in the 100 MIU/kg/d dose group during weeks 2 and 4 on study. There were no significant changes in erythrocyte or platelet counts, hemoglobin or hematocrit, MCV, MCH, or MCHC in the groups of animals treated with either 4 or 20 MIU/kg/d IFN, as compared to the saline control group at any time point on study.

Serum biochemistry profiles were within normal limits for all control and low and mid-dose, IFN-treated animals through the duration of treatment, with the exception of a single incidence of elevated ALT in one female monkey (animal #15F) in the group treated with 4 MIU/kg/d at week 13. The two female monkeys in the high-dose group that were sacrificed early both had changes in clinical chemistry prior to sacrifice. Monkey #31F had decreased total protein and an elevation in transaminases prior to sacrifice, and monkey #29F had decreased total protein. Transient decreases in serum albumin and/or total protein were also noted at weeks 2 and 4 in 4/6 surviving animals in the group treated with 100 MIU/kg/d IFN.

At necropsy, both female monkeys that were sacrificed early due to toxicities had apparent decreases in total body fat, and both macroscopic and microscopic evidence of hemorrhage, mononuclear cell infiltrates, and fibrosis and regeneration at the injection site. Monkey #29F had dark red foci present in the lung on gross evaluation, which microscopically were correlated with focal to diffuse areas of hemorrhage in the alveolar walls. Sporadic areas of hepatocellular necrosis, hyaline deposition, and Kupffer cell hypertrophy and hyperplasia were present in the liver of this monkey on microscopic evaluation. Kupffer cell hypertrophy and hyperplasia, as well as hepatocyte vacuolization in the centrilobular regions and occasional single cell necrosis were also present in the liver of monkey #31F at sacrifice.

Pathologic findings in the surviving animals consisted mainly of discoloration at the injection site on gross evaluation, which was correlated with hemorrhage, inflammatory cell infiltrates, fibrosis, and evidence of muscle cell regeneration on histologic examination. These findings were present in 2/8 vehicle control monkeys, and in 4/8, 5/8, and 3/6 surviving monkeys in the groups treated with 4, 20, or 100 MIU/kg/d IFN, respectively. All other macroscopic and microscopic findings were considered by the reviewing pathologist to be incidental to treatment with the test article.

Anti-interferon neutralizing antibody activity was detected in serum samples from 5/8 monkeys each in the groups treated with 4 or 20 MIU/kg/d IFN, and in 3/6 surviving monkeys in the high-dose group at weeks 4 and 13 after dosing. These results are confounded, however, by the presence of IFN in the samples from the high-dose monkeys, which may have bound with antibody present and limited the detection of neutralizing activity. Antibody titers were positive for neutralizing activity in samples from 4/8 low-dose monkeys and 1/8 mid-dose animals at week 13, even when the serum samples were diluted 1:100.

In summary, treatment of cynomolgus monkeys with IFN for three months was associated with transient inappetence, decreases in erythrocyte parameters and platelet counts, increases in APTT, and elevations in serum hepatic transaminases. Monkeys in all groups, including the saline control, developed inflammation and hemorrhage at the injection site. Development of serum neutralizing antibody activity occurred by 4 weeks in approximately 50% of the IFN-treated animals, and may have limited the toxicities observed. Based on the clinical and hematologic toxicities, the NOAEL for IFN in this species is 4 MIU/kg/d, by i/m injection, for 91 to 92 days of exposure.

Study #85026 (——— Study #SCH01). ***Reproductive toxicity of SCH 30500 in Rhesus monkeys (Macaca mulatta).***

Comment: This study was previously submitted and reviewed under PLA #89-0381. A brief summary of the results is provided here, for reference purposes only.

The reproductive effects of IFN were evaluated in 48 pregnant, female Rhesus macaques. Twelve animals per group were treated daily from GD20 until GD80 with either vehicle control (0.15% sterile saline), 7.5, 15, or 30 MIU/kg/d IFN, by i/m injection. Significant fetal losses were observed in 9/12 animals treated with 30 MIU/kg/d IFN ($p \leq 0.005$, ANOVA) and in 5/12 monkeys treated with 15 MIU/kg/d IFN ($p \leq 0.05$, ANOVA), occurring between GD28 and GD78 (days 8 through 58 of treatment), as compared to the control group of monkeys. Evaluation of the aborted fetuses did not reveal any evidence of gross or skeletal malformations.

In the remaining animals, pregnancies were carried to term in 11/12 control monkeys, 10/12 animals treated with the lowest dose of IFN, 7/12 monkeys treated with 15 MIU/kg/d IFN, and 3/12 monkeys treated with the highest dose of IFN. Pregnancies were terminated by Caesarean section at GD 100, and the fetuses evaluated for standard teratologic parameters, including physical dimensions, weight of the fetus and placenta, gross abnormalities, and Alizarin Red S staining for skeletal defects. No gross malformations, nor abnormalities in the placentas were observed in the fetuses in either the control or any of the IFN-treated groups, with the exception of non-viable monochorial diamniotic twins in one animal (monkey #MMY 16880) in the mid-dose group. One of the fetuses was an acardius acephalus, and was considered to be of spontaneous origin and not related to the treatment. Examination of the fetal skeletons did not reveal any treatment-related abnormalities. Incidental findings included the presence of a thirteenth rib in one fetus each from animals in the control, mid-dose, and high-dose IFN groups, and extra cervical rib pairs in two fetuses each from the low- and high-dose IFN groups and one fetus from a monkey in the mid-dose IFN group. Two animals were found to have anatomical variations in the facial bones, including one fetus from the vehicle control group and one fetus from a monkey treated with 7.5 MIU/kg/d WFN. An additional fetus from a dam in the group treated with 15 MIU/kg/d had a suture projecting dorsal-medially in line with the linea temporalis superior and ending at the lambda suture. These anatomical variations were all considered incidental to treatment with IFN.

Analysis of serum progesterone from a subgroup of the last seven pregnant animals entered on the study (1 control, 2 low-dose, 2 mid-dose, and 2 high-dose IFN-treated) indicated that the levels of the hormone between GD28 and GD34 were within normal limits for pregnant Rhesus monkeys, with the exception of one animal each in the groups treated with 15 or 30 MIU/kg/d IFN. Monkey #MMU 17078 (mid-dose group) showed decreased serum progesterone levels on GD30, while animal #MMU 18015 had decreased serum progesterone on GD25, GD28, and GD30. Both animals spontaneously aborted (as detected by loss of viable fetus on ultrasound) on GD 30.

In summary, treatment of pregnant female Rhesus monkeys with IFN was associated with a dose-related increase in maternal toxicity including inappetence and weight loss, diarrhea, constipation, depression of activity, epistaxis, and/or nasal discharge. Dose-related increases in embryonic/fetal losses were also observed, and appear to be related to decreases in serum progesterone levels. The NOAEL for IFN in pregnant, female macaques is 7.5 MIU/kg/d, when administered by i/m injection from GD20 to GD80.

Study #98448 — Study # ——— *Acute subcutaneous toxicity study of SCH215600 in mice.*

The acute toxicity of the polyethylene glycol (PEG) used in the PEG-IFN conjugate was evaluated in ICR mice after a single s/c injection. Five mice per sex per group were injected with either vehicle (placebo for SCH 215600 PEG, agent not specified), or 6480 $\mu g/m^2$ PEG on day 1, and observed for 14 days. There were no clinical toxicities, alterations in general condition, body weight, or food consumption over the 14 d duration of the study. In summary, the LD_{50} for SCH 215600 PEG was > 6480 $\mu g/m^2$ after a single, s/c injection in ICR mice.

Study #98449 — Study # ——— *Acute intravenous toxicity study of SCH215600 in mice.*

The acute toxicity of the polyethylene glycol (PEG) used in the PEG-IFN conjugate was evaluated in ICR mice after a single i/v injection. Five mice per sex per group were injected with either vehicle (placebo for SCH 215600 PEG, agent not specified), or 6480 $\mu g/m^2$ PEG on day 1, and observed for 14 days. There were no clinical toxicities, alterations in general condition, body weight, or food consumption over the 14 d duration of the study. In summary, the LD_{50} for SCH 215600 PEG was > 6480 $\mu g/m^2$ after a single, i/v injection in ICR mice.

Study #98446 — Study # ——— *Acute subcutaneous toxicity study of SCH215600 in rats.*

The acute toxicity of the polyethylene glycol (PEG) used in the PEG-IFN conjugate was evaluated in outbred, Sprague-Dawley rats after a single s/c injection. Five rats per sex per group were injected with either vehicle (placebo for SCH 215600 PEG, agent not specified), or 6480 $\mu g/m^2$ PEG on day 1, and observed for 14 days. There were no clinical toxicities, alterations in general condition, body weight, or food consumption over the 14 d duration of the study. In summary, the LD_{50} for SCH 215600 PEG was > 6480 $\mu g/m^2$ after a single, s/c injection in Sprague-Dawley rats.

Study #98447 — Study # ——— *Acute intravenous toxicity study of SCH215600 in rats.*

The acute toxicity of the polyethylene glycol (PEG) used in the PEG-IFN conjugate was evaluated in outbred, Sprague-Dawley rats after a single i/v injection. Five rats per sex per group were injected with either vehicle (placebo for SCH 215600 PEG, agent not specified), or 6480 $\mu g/m^2$

PEG on day 1, and observed for 14 days. There were no clinical toxicities, alterations in general condition, body weight, or food consumption over the 14 d duration of the study. In summary, the LD_{50} for SCH 215600 PEG was > 6480 $\mu g/m^2$ after a single, i/v injection in Sprague-Dawley rats.

Study #SN98222 ——Study # ———). *A 13-week subcutaneous toxicity study of SCH 215600 in rats.*

The safety of the methoxy-polyethylene glycol moiety SCH 215600 used in the PEG-IFN conjugate was evaluated in Sprague-Dawley rats in a repeat-dose toxicity study. Ten rats per sex per group were treated with either saline or SCH 215600 placebo (composition net specified), or 45.5, 455, or 1138 $\mu g/m^2$ SCH 215600 by s/c injection, twice weekly for 13 weeks. An additional five rats/sex were treated with either the placebo control or 1138 $\mu g/m^2$/dose PEG for 13 weeks, then held for a 4 week, treatment-free recovery period, to determine the reversibility of any treatment-related toxicities. Animals were observed daily for clinical signs of toxicity, and detailed physical examinations, individual body weights, and food consumption were determined weekly. Samples of peripheral blood were obtained at weeks 3, 11, and 16 on study and at terminal sacrifice at either week 13 or week 17 for the determination of serum biochemistry and hematology profiles. Urinalysis was performed at weeks 3, 11, and 12. Ophthalmologic examinations were conducted on all animals at baseline, prior to the initiation of dosing, and at weeks 3, 12, and 16 on study. At terminal sacrifice, complete necropsies and evaluation of gross pathologic findings were performed for each animal. Histologic evaluation of tissue samples obtained at the week 13 necropsy only was performed for animals in both the saline and placebo control groups, rats treated with 1138 $\mu g/m^2$/dose PEG, and any animal with a gross lesion present on macroscopic evaluation of pathology.

All animals in both the control and the PEG-treated groups survived until terminal sacrifice. There were no overt clinical signs of toxicity noted, and no treatment-related clinical observations. No adverse effects of PEG treatment were noted on total body weights, body weight gain, or food consumption over the duration of the study. No ophthalmologic toxicities were noted in either the control or the SCH 215600-treated animals. During the treatment period, there were no effects on urinalysis profiles that were related to administration of the test article. At the week 16 recovery time point, however, there was an increase in mean total urine volume and a decrease in mean urine osmolality in the group of female rats treated with 1138 $\mu g/m^2$/dose SCH 215600, as compared to the placebo control group. However, this change was due to increased urine output by one rat (animal #99798F), and no similar effect was observed in the male animals. This finding was considered incidental to treatment with the test article, since it was not observed during the dosing period, and no similar findings were observed in the male animals in this same group.

There were sporadic changes in hematologic profiles in both mean group values and individual animals in both the PEG-treated and control groups of rats over the duration of the study. Mean group values for total white cell counts, erythrocyte counts, hemoglobin levels and hematocrit were increased in male animals in all groups at week 11 on study, as compared to the week 3 or the recovery time points. There were no apparent relationships to the dose of the test article in either the incidence or severity of these changes. Similar increases were observed in female rats across all dose groups at the week 11 time point, with the exception of total leukocyte counts. A slight, although statistically not significant decrease in total white cell count as compared to both the placebo and saline control groups was observed for female rats treated with 1138 $\mu g/m^2$/dose SCH

215600 at the week 11 time point. This finding remained decreased at the week 16 recovery time point for animals in this dose group, however, the mean value was within the normal range for leukocyte counts in Sprague-Dawley rats, so the clinical significance is unknown.

There were no treatment-related effects observed on the total platelet counts, coagulation profiles (PT and APTT), or leukocyte differential counts. There was an increase in the mean percentages of both polymorphonuclear neutrophils and monocytes, with a concomitant decrease in the percentage of lymphocytes noted for all groups of animals at week 11 as compared to week 3 on study. The differential counts at the week 16 recovery time point had decreased to approximately the week 3 values in male rats in the placebo group, but not in the high-dose male rats or either group of female animals. The clinical significance of this finding is unknown.

There were no statistically significant differences in serum biochemistry profiles between saline and placebo control, and SCH 215600-injected rats during the course of the treatment period. Mean values for serum triglyceride levels were elevated in the group of male rats treated with 1138 μg PEG/m^2/dose at the week 16 recovery time point, as compared to the placebo control group. However, this finding was not observed during the treatment with the test article, and its relationship to dosing with PEG is unknown.

At necropsy, there were no detectable effects of SCH 215600 treatment on either absolute or relative organ weights, or macroscopic organ pathology. Incidental findings included enlarged mediastinal and/or bronchial lymph nodes in rats of both sexes in all groups, including the controls at the week 13 sacrifice, without relationship in either incidence or severity to the dose of test article. The lymph node enlargement was not observed in either group of animals after the 4 week recovery period. Other incidental findings included single incidences each of small testes/epididymides, hepatocellular necrosis, exostosis of the femur, pancreatic abscess, and cysts in the prostate and spleen in male rats, and enlarged mandibular lymph nodes in one female rat in the low dose group. Similar findings have previously been reported for this strain of rat, and are not considered unusual or unexpected.

There was a high incidence of pulmonary findings on microscopic evaluation of lung samples from both male and female animals in all dose groups, including both the saline and the placebo controls. Pulmonary changes included minimal to mild, multifocal, predominantly mononuclear cell and/or alveolar macrophage infiltrates in both the perivascular regions as well as in the alveolar interstitia. The severity of the findings was similar across all dose groups, and there was no apparent relationship in the incidence of animals affected to the dose of SCH 215600. Similar findings in the lungs of laboratory rats have previously been described[7]. Other, incidental findings on histologic examination included minimal to moderate, focal areas of lymph node hyperplasia, minimal to mild, multifocal areas of hepatocellular vacuolization in both the mid-zonal and periportal regions of the lobule, mild to moderate areas of mineralization in the kidney tubules, cortex, and renal papillae, and areas of minimal to mild, mononuclear cell infiltrates and fatty necrosis in the pancreas. These changes were present in approximately equal incidence in severity in both control groups, as well as the SCH 215600-treated rats, and were considered by the reviewing pathologist to be unrelated to treatment with the test article.

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[7] Ewell, M.R., J.F. Mahler, and G.N. Rao. 1997. Inflammatory lesions in the lungs of rats. *Toxicol. Pathol.*, **25**:529-531.

In summary, treatment of rats for 13 weeks with SCH 215600 PEG by daily, s/c injection was not associated with any toxicity related to the test article. The NOAEL for PEG in this species is 1138 $\mu g/m^2$/dose, or a cumulative, weekly dose of 2276 $\mu g/m^2$.

Study #SN98223 (— Study #' ———). *A 13-week subcutaneous toxicity study of SCH 215600 in cynomolgus monkeys.*

The safety of the methoxy-polyethylene glycol moiety SCH 215600 used in the PEG-IFN conjugate was evaluated in male and female cynomolgus monkeys in a repeat-dose toxicity study. Three monkeys per sex per group were treated with either saline or SCH 215600 placebo (composition not specified), or 45.5, 455, or 1138 $\mu g/m^2$ SCH 215600 by s/c injection, twice weekly for 13 weeks. An additional two monkeys/sex were treated with either the placebo control or 1138 $\mu g/m^2$ PEG for 13 weeks, then held for a 4 week, treatment-free recovery period, to determine the reversibility of any treatment-related toxicities. Animals were observed twice daily for clinical signs of toxicity, and food consumption was recorded daily. Detailed physical examinations, including individual body weights were determined weekly, and selected physiologic parameters (heart rate, respiratory rate, rectal body temperature, and blood pressure were recorded at baseline, and at weeks 4, 13, and 16 on study. Samples of peripheral blood were obtained at weeks 3 or 4, 11, and 16 for the determination of serum biochemistry and hematology profiles. Urinalysis was performed at weeks 3 or 4, 11, and 16. Ophthalmologic examinations were conducted on all animals once prior to the initiation of dosing, and at weeks 3, 12, and 16 on stud, and electrocardiograms were performed at weeks 4, 13, and 16. At terminal sacrifice, complete necropsies and evaluation of gross pathologic findings were performed for each animal. Histologic evaluation of tissue samples obtained at the week 13 necropsy only was performed for animals in both the saline and placebo control groups, and the monkeys treated with 1138 $\mu g/m^2$ PEG/dose.

There were no deaths on study, and all monkeys in both control groups and the PEG-treated groups survived until terminal sacrifice at weeks 13 or 17. There were no overt clinical signs of toxicity noted at any dose level of SCH 215600. Several male monkeys in the group treated with 1138 $\mu g/m^2$ PEG/dose developed diarrhea, mucoid feces, and/or mucoid feces with red material present at various time points during the duration of the study. However, the majority of the reported findings were for monkey #980M, which had several events reported during both the treatment and recovery periods. No similar changes were noted in either the saline or placebo control groups, nor in the female monkeys treated with SCH 215600.

No adverse effects of PEG treatment were noted on total body weights, body weight gain, or food consumption over the duration of the treatment and recovery periods. There were no remarkable effects of SCH 215600 treatment on heart rate, body temperature, respiratory rate and blood pressure, as compared to the saline or placebo control animals. No ophthalmologic toxicities or alterations in electrocardiograms were noted in either the control or the SCH 215600-treated animals at any time point on study. There were nor remarkable differences in urinalysis profiles in the monkeys receiving SCH 215600, as compared to either the saline or placebo control groups.

Hematology and serum biochemistry profiles were comparable to the saline control group in the monkeys treated with SCH 215600 or SCH 215600 placebo. There were no placebo or test article related findings on macroscopic evaluation of pathology at necropsy, and no significant differences in mean organ weights between the PEG-treated and the placebo or saline control groups.

Incidental findings at the 13 week sacrifice included single animals with small testes and/or epididymides, nodules and/or cysts on the ovary or oviduct, an accessory spleen in one female monkey in the placebo control group, and thyroid cysts in 1 male monkey each in the saline and placebo control groups, and one female monkey in the group treated with 1138 μg PEG/m^2/dose. After the 4 week recovery period, incidental findings included one placebo control male monkey with an enlarged spleen, and enlarged and/or reddened femoral, mediastinal, iliac, and renal lymph nodes, and ovarian cysts in one female monkey from the high-dose group.

Inflammatory infiltrates of minimal to mild severity were present on microscopic examination of the injection site in 2/6 placebo control, 4/6 saline control, and 4/6 high-dose monkeys at the end of the 13 week treatment period. There were no treatment-related differences in the severity of these findings between the two control groups and the monkeys treated for 13 weeks with 1138 μg SCH 215600/m^2/dose. Cellular infiltrates of minimal to mild severity were also noted in the brain, liver, lungs, and heart of animals in the saline and placebo control groups, and in one monkey (animal #974M) in the high-dose group. Other incidental findings included minimal, focal areas of mineralization in the kidney medulla, vacuolization of renal tubular cells, hepatocytes, and adrenal cortical cells, and areas of minimal to mild, vacuolization and mononuclear cell infiltrates in the pancreas in one placebo control female (animal #997F) and two female monkeys in the group treated with 1138 μg/m^2/dose SCH 215600. These changes were present in approximately equal incidence in severity in both control and the PEG-treated groups, and were considered by the reviewing pathologist to be unrelated to treatment with the test article.

In summary, treatment of cynomolgus monkeys for 13 weeks with SCH 215600 PEG by daily, s/c injection was not associated with any toxicity related to the test article. The NOAEL for PEG in this species is 1138 μg/m^2/dose, or a weekly cumulative dose or 2276 μg/m^2.

Study #SN 98459 —— Study # ———). *Embryo-fetal developmental toxicity study of SCH 215600 by subcutaneous injection in rats.*

The developmental toxicity of the methoxy-polyethylene glycol moiety of PEG-IFN (SCH 215600, PEG) was determined in Sprague-Dawley rats. Twenty-five pregnant, female rats were dosed on GD6 through GD17 with vehicle control (SCH 215600 placebo), or 113, 400, or 800 μg/m^2 SCH 215600 (PEG) by s/c injection. All animals were examined daily for evidence of clinical toxicities, and food consumption and body weights were recorded on days 0, 6, 9, 12, 15, 17, and 20 after mating. On GD20, all rats were euthanized, fetuses harvested by Caesarean section, and examined for any gross anomalies. The numbers of corpora lutea, implantation sites, live and dead fetuses, and resorptions were counted and the distribution of fetuses in the uterus was determined for each animal. Approximately one half of the fetuses were placed in Bouin's fixative and evaluated for soft-tissue anomalies, while the remainder were fixed and stained with Alizarin Red S for evaluation of skeletal malformations.

There was no evidence of maternal toxicity in the placebo control group, or at any dose of SCH 215600 tested. All animals gained weight throughout gestation, and net body weights, gravid uterine weights, and food consumption were comparable between all groups. There were no treatment-related effects of SCH 215600 on maternal organ pathology at scheduled necropsy. Incidental findings, including a cystic ovary in animal #97150F in the 400 μg/m^2/d dose group and fused placentae (sites #16 and #17) in rat #97209 in the high-dose group. Two animals in the

group treated with 113 μg/m^2/d SCH 215600 also had findings which were considered incidental to treatment with the test article; pale areas on the liver were present in animal #97170F, and rat #97191F had enlarged placentae at sites #3, #8, and #12. All other animals had normal findings on gross pathologic evaluation.

Reproductive parameters were not affected by treatment with SCH 215600. Twenty-two of 25 control, 25/25 low-dose, 24/25 mid-dose, and 23/25 high-dose rats were gravid at terminal sacrifice. There were no statistically significant differences in the mean numbers of corpora lutea, implantation sites, or resorptions between the control and the groups of animals treated with either 113 or 400 μg/m^2/d PEG. No differences in implantation site or resorption numbers as compared to the placebo control group were noted for animals in the 800 μg/m^2/d dose group. However, the mean number of corpora lutea for this dose group was significantly increased over the control group($p \leq 0.05$, Dunnett's test). The toxicologic significance of this finding is unknown.

There were no treatment related effects of SCH 215600 on the either the total number or the number of viable fetuses, fetal sex distribution, or fetal body weights as compared to the placebo control group. No gross external, visceral, or skeletal malformations were observed at any dose level of SCH 215600, with the exception of a *situs inversus* in one fetus from a dam treated with 113 μg/m^2/d PEG (fetus #97206-12). Skeletal variations including cervical ribs, unossified sternebrae, and/or rudimentary ribs occurred at similar incidence in the control and SCH 215600-treated groups, and were considered normal variations in fetal development for this strain of rats.

In summary, treatment of pregnant female Sprague-Dawley rats with daily, s/c injections of SCH 215600 PEG from GD6 to GD17 had no adverse effects on maternal health, reproductive parameters, or fetal development. The NOAEL for SCH 215600 under the conditions of this study is 800 μg/m^2/d.

Study #98291 (Report #P-7028). ***Embryo-fetal developmental toxicity study of SCH 215600 by subcutaneous injection in rabbits.***

The developmental toxicity of the methoxy-polyethylene glycol moiety of PEG-IFN (SCH 215600, PEG) was determined in New Zealand white rabbits. Twenty pregnant, female rabbits were dosed on GD7 through GD19 with vehicle control (SCH 215600 placebo), or 113, 400, or 800 μg/m^2 SCH 215600 (PEG) by s/c injection. All animals were examined daily for evidence of clinical toxicities, and food consumption and body weights were recorded on days 0, 7, 10, 13, 16, 19, 22, 25, 28 and 30 after mating. On GD30, all rabbits were euthanized, fetuses harvested by Caesarean section, and examined for any gross anomalies. The numbers of corpora lutea, implantation sites, live and dead fetuses, and resorptions were counted and the distribution of fetuses in the uterus was determined for each animal. Approximately one half of the fetuses were placed in Bouin's fixative and evaluated for soft-tissue anomalies, while the remainder were fixed and stained with Alizarin Red S for evaluation of skeletal malformations.

There was no evidence of maternal toxicity in the placebo control group, or at any dose of SCH 215600 tested. The only clinical observation noted was stained, fur, which occurred in all groups of animals including the controls. The incidence of stained fur was 3/20, 2/20, 10/20, and 3/20 for rabbits in the control, 113, 400, and 800 μg/m^2/d dose groups, respectively. All animals gained weight throughout gestation, and net body weights, gravid uterine weights, and food consumption

were comparable between all groups. There were no treatment-related effects of SCH 215600 on maternal organ pathology at scheduled necropsy.

Reproductive parameters were not affected by treatment with SCH 215600. Eighteen of 21, 20/21, 18/20, and 18/20 rabbits were gravid at terminal sacrifice in the placebo control, 113, 400, and 800 $\mu g/m^2/d$ dose groups, respectively. There were no differences in the mean numbers of corpora lutea, implantation sites, or resorptions, pre- or post-implantation losses, or gravid uterine weights between the control group and animals treated with SCH 215600 at any dose level. There were no placental observations that were related to treatment with PEG.

There were no treatment related effects of SCH 215600 on the either the total number or the number of viable fetuses, fetal sex distribution, or fetal body weights as compared to the placebo control group. No gross external or visceral malformations were observed at any dose level of SCH 215600, with the exception of an omphalocele (midline defect with intestine and viscera protruding) in one fetus from a dam in the placebo control group (fetus #9-1). Skeletal malformations (hemivertebrae) were present in 1 and 2 fetuses from the 400 and 800 $\mu g/m^2/d$ dose groups, respectively, and were not considered to be related to SCH 215600 treatment.

Comment: The sponsor reports that for rabbits of this strain in this facility, the historical control incidence of hemivertebrae is 2.9% of litters, or 3/865 (0.3%) of fetuses. The incidence of hemivertebrae on the present study was 3/74 fetuses, or 0.4%, and is felt to be within the limits of the historical controls.

No external or gross visceral variations were identified in fetuses from any group of rabbits treated with SCH 215600 PEG. In the placebo control group, one fetus had a dilated renal pelvis. Skeletal variations included fused sternebrae in 3 fetuses (2/18 litters) only in the group of animals treated with 400 $\mu g/m^2/d$ PEG. Additionally, one fetus in this same dose group had an extra rib (unassociated with the vertebral arch), as well as hemivertebrae. These findings were considered incidental to treatment with SCH 215600. All remaining skeletal variations, including extra thoracic ribs (single or pairs), decreased or unossified sternebrae, distal humeral and/or femoral epiphyses, and/or coracoid processes, and/or rudimentary ribs occurred at similar incidence in the control and SCH 215600-treated groups, and were considered normal variations in fetal development for this strain of rabbits.

In summary, treatment of pregnant female New Zealand white rabbits with daily, s/c injections of SCH 215600 PEG from GD7 to GD19 had no adverse effects on maternal health, reproductive parameters, or fetal development. The NOAEL for SCH 215600 under the conditions of this study is 800 $\mu g/m^2/d$.

Study #99331. ***Effect of SCH 54031 (PEG_{12000}-IFN) on the menstrual cycle and estradiol and progesterone levels in cynomolgus monkeys.***

The effects of treatment with PEG-IFN on hormonal status and menstrual cyclicity were evaluated in non-pregnant cynomolgus monkeys. Thirty-two sexually mature, female monkeys (age range not specified; weight 2.1 to 4.8 kg) were selected for study after pre-treatment screening for estradiol and progesterone levels indicative of regular menstrual cyclicity. Pre-test blood samples for hormone analysis were collected on the first day of the first menstrual cycle (cycle 1), then

every third day thereafter. The first day of dosing with PEG-IFN or control article(s) was the first day of the second menstrual cycle, at doses of 0 (vehicle control; 0.9% normal saline), 52, 262, or 4239 μg/m^2 PEG-IFN, or 3105 μg/m^2 IFN (INTRON®-A) as a positive control. All animals were administered the test article by s/c injection once every other day for the duration of one menstrual cycle, or to a maximum of 23 doses of test material (day 45 on study); the number of doses administered ranged from 13 to 23. Animals were followed for at least one menstrual cycle following the completion of the dosing period, or to a maximum of 66 days, until menstrual cyclicity had resumed to normal. Hematology profiles were determined from blood samples obtained on days 0 of cycle 2 (prior to initiation of dosing), and on days 7, 15, and 29 of cycle 2 and days 14 and 28 of cycle 3 (post-treatment recovery period).

Two monkeys in the group treated with INTRON®-A exhibited abnormal clinical signs, including inappetence, decreased body weight, changes in stool composition, and hypothermia which were associated with toxic effects of IFN treatment. Female monkey #32F was sacrificed for humane reasons on day 25 of study following a decrease in food consumption, progressively decreased body weight, dehydration, hypoactivity, hypothermia, and a hunched postural appearance. A gross evaluation of pathology was performed at necropsy, and failed to detect any pathologic cause for the animal's deterioration. Similar clinical signs were noted in female #26F, with abnormal posturing noted on days 24-29 on study. Dosing with INTRON®-A was terminated in this animal after the d 28 dose; by day 36 on study, no adverse clinical signs were noted in this monkey and it remained on study until completion.

All other animals treated with either the saline control or PEG-IFN survived the treatment and recovery periods with no deaths or overt clinical signs of toxicity. Minor clinical signs noted included scabbing and abrasions at the injection site(s), alopecia, nasal discharge, scant feces, and a prolapsed uterus, and occurred sporadically with no relationship in either incidence or severity to the dose of PEG-IFN administered. Decreases in food consumption by approximately 50% of baseline were noted beginning during the second week of treatment in 3/7, 2/7, and 5/7 of the monkeys treated with 52, 262, and 4239 μg/m^2 PEG-IFN, and in 6/7 monkeys treated with the INTRON®-A positive control. In all cases, food consumption returned to normal by the end of the treatment period, and the animals had all gained weight as compared to baseline at the end of the study period. A transient decrease in body weights was noted in 6/7 female monkeys in the highest dose group beginning during the second week of dosing and persisting throughout the dosing period; however, one monkey in this group (animal #12F) actually gained body weight during the dosing period.

Hematology analyses demonstrated no remarkable differences in any of the erythrocyte parameters, as compared to either baseline values or to the saline controls that could be attributed to treatment with PEG-IFN. Slight, although statistically not significant decreases in red cell numbers, hematocrit, and hemoglobin concentration were noted in the mean values for the monkeys treated with the highest dose of PEG-IFN at the end of the dosing period as compared to baseline or saline controls. Additionally, an approximate two-fold increase in reticulocyte counts as compared to baseline or saline control groups was also observed at this time point only in this group of animals, suggesting that PEG-IFN may have had a slight effect on erythrocyte turnover. There were no significant nor biologically relevant changes in platelet counts for any of the groups of animals during the PEG-IFN or INTRON®-A treatment periods, with the exception of a 25% decrease in platelet counts in the highest dose group at week 2, as compared to baseline (not statistically significant). This effect rapidly recovered and the platelet counts for this group of monkeys had

returned to near baseline at the following time point (week 3 of treatment). Total leukocyte, as well as absolute neutrophil counts were decreased by 30 to > 65% in a dose-related fashion at week 2 (day 7) of treatment as compared to baseline, in all groups of monkeys treated with PEG-IFN, as well as in monkeys treated with the INTRON®-A positive control. Leukocyte and PMN counts remained decreased in all interferon-α-treated groups at week 3 (day 15) of treatment, but had recovered to baseline values by the end of the treatment period at day 29. Hematologic profiles were not determined in either the pre-treatment or post-dosing evaluation periods. Serum biochemistry parameters were not determined in this study.

Since individual animals were found to menstruate at different calendar dates, the data were normalized by selecting the first day of bleeding of the second (treatment) cycle as day 0, then ordering the other samples at daily intervals before and after this time point. If animals had not begun menstruating by d 45 on study (after 23 treatments with IFN), treatment was discontinued, and the following day was considered recovery day +1.

There were no significant differences in the mean length of menstrual cycles between baseline, treatment, and post-treatment cycles in animals treated with either the vehicle control, 52, or 262 mg/m^2 PEG-IFN/dose (Table X, below). By contrast, animals in the groups treated with either 3105 μg/m^2 INTRON®-A or 4239 μg/m^2 PEG-IFN demonstrated delayed menstruation during the treatment cycle with interferon. Mean values for menstrual cycle duration in monkeys receiving the saline control were 32.5 ± 7.1 days during treatment, while animals treated with INTRON®-A or 4239 μg/m^2 PEG-IFN/dose had mean cycle durations of 42.1 ± 7.6 days and 41.0 ± 8.9 days, respectively, during the treatment cycle. Although there were no statistically significant differences evident in the mean values for each group, there was a dose-related increase in the frequency of individual cycles falling outside of the normal range (<24 or >38 d). Of the thirty-two animals monitored during the treatment period, 1/4 in the saline control group, 1/7 monkeys each in the low- and mid-dose PEG-IFN groups, and 6/7 animals each in the INTRON®-A and high-dose PEG-IFN treated groups were abnormal in cycle duration. The data are presented in the table below.

Table X - Menstrual Cyclicity in Cynomolgus Monkeys after PEG-IFN Administration

Animal Number	Dose Group	Cycle Duration
29	Saline control	28 d
30		43 d
4		29 d
13		30 d
32	3105 μg/m^2 INTRON®-A	> 45 d[a]
26		> 45 d
27		> 45 d
35		> 45 d
15		> 45 d
11		> 45 d
1		> 45 d
21	52 μg/m^2 PEG-IFN	30 d
9		27 d
18		29 d
5		36 d
8		34 d
33		29 d
28		39 d
3	262 μg/m^2 PEG-IFN	36 d
23		39 d
17		29 d
19		31 d
20		29 d
31		36 d
25		30 d
12	4239 μg/m^2 PEG-IFN	41 d
10		> 45 d[a]
24		> 45 d
6		> 45 d
34		> 45 d
16		> 45 d
7		21 d

[a] dosing with INTRON®-A or PEG-IFN stopped on d 45 to allow recovery of menstrual cycle

Subcutaneous injection of PEG-IFN at 4239 μg/m^2/dose also resulted in changes in hormonal status, which were related to the irregularities in menstrual cyclicity. In general, peak serum progesterone, and estradiol were not significantly changed from pre-treatment in animals treated with either the vehicle control or the 52 or 262 μg/m^2/dose PEG-IFN dose levels. Every other day s/c injection of either INTRON®-A or PEG-IFN at the highest dose level resulted in a blunting of peak serum estradiol, and progesterone levels during the treatment cycle, as compared to during cycle 1. Mean, peak values for the two reproductive hormones are presented in Table XI, below:

Table XI - Peak Hormonal Levels in Non-Pregnant Female Cynomolgus Monkeys after Treatment with PEG-IFN

Group	Mean Peak Estradiol (pg/ml) ± SE			Mean Peak Progesterone (ng/ml), ± SE		
	Cycle 1	Treatment Cycle 2	Cycle 3	Cycle 1	Treatment Cycle 2	Cycle 3
Control	141 ± 51	89 ± 29	94 ± 53	9.1 ± 2.7	6.8 ± 2.6	10.3 ± 8.8
INTRON®-A	167 ± 60	40 ± 28	107 ± 54[a]	7.1 ± 1.4	2.8 ± 1.6	4.4 ± 2.6
52 μg/m² PEG-IFN	116 ± 63	125 ± 52	115 ± 38	9.5 ± 1.7	6.6 ± 1.7	9.4 ± 1.4
262 μg/m² PEG-IFN	85 ± 22	87 ± 32	152 ± 46	7.7 ± 1.6	6.5 ± 2.9	9.2 ± 2.3
4239 μg/m² PEG-IFN	113 ± 25	103 ± 58[b]	85 ± 32[c]	6.8 ± 1.2	3.8 ± 1.0	4.7 ± 1.8

[a] biphasic peak seen, with first peak of 107 ± 54 pg/ml observed at day 21 of recovery and second peak of 150 ± 73 pg/ml observed at d 42 on recovery
[b] biphasic peak seen, with first peak of 103 ± 58 pg/ml observed at day 33 on treatment and second peak of 75 ± 29 pg/ml observed at d 45 on treatment
[a] biphasic peak seen, with first peak of 85 ± 32 pg/ml observed at day 33 of recovery and second peak of 237 ± 227 pg/ml observed at d 51 of recovery

In summary, treatment of non-pregnant, female cynomolgus monkeys with either 3105 μg/m²/dose INTRON®-A or 4239 μg/m²/dose PEG-IFN inhibited ovarian function during the administration period in 6/7 monkeys each in the two dose groups, however, recovery was observed to normal levels in the following cycle. The effects on fertility were evidenced by decreases in serum estradiol and progesterone levels in all animals treated in these two dose groups during the administration period, and by irregularities in the duration of menstrual cycle, which were dose-related in incidence. The NOAEL for PEG-IFN effects on reproductive function is 262 μg/m²/dose in cynomolgus monkeys. The mechanism by which IFN exerts negative effects on ovarian function cannot be determined from these studies.

SUMMARY AND CONCLUSION:

The safety, biochemical, and pharmacokinetic activities of PEG-IFN and its components SCH 215600 PEG and INTRON®-A were evaluated in ICR mice, Sprague-Dawley rats, New Zealand white rabbits, and cynomolgus and Rhesus monkeys *in vivo*. Pharmacokinetic studies in rats and cynomolgus monkeys demonstrated similar absorption and elimination profiles of PEG-IFN between the two species after i/v injection, with an approximate $t_{½elim}$ of 25 to 26 h. Systemic exposure in rats after i/v injection, as calculated from the $AUC_{(0-\infty)}$ was increased in a dose-related fashion, was approximately linear, and was similar to that observed in cynomolgus monkeys treated by either i/v or i/m injection. Bioavailability by the s/c route in both rats and cynomolgus monkeys was between 50 and 100%.

PEG-INTRON™ has pharmacologic and toxicologic profiles similar to other type I interferons. Major findings in cynomolgus monkeys after repeated, every other day, s/c dosing with PEG-IFN for 4 weeks at 1414, 4239, or 14,130 μg/m^2/dose included decreased food consumption in the absence of significant weight loss, slight to moderate decreases in erythrocyte parameters, platelet and leukocyte counts, and transient elevation in hepatic transaminases (ALT, AST). No similar changes in appetite, total body weight gain, or hematologic profiles were noted in CD-1(ICR) mice or Sprague-Dawley rats receiving a single i/v or s/c injection of PEG-IFN at doses of up to 60,410 μg/m^2. The decrease in platelets and leukocytes observed in cynomolgus monkeys were related to the dose of PEG-IFN administered, and were only evident during the second and third weeks of treatment. All changes were reversible by the end of the treatment period, with the exception of the red cell losses in several animals, and were correlated with development of anti-interferon neutralizing activity in the serum. Mild to moderate, local irritation and/or inflammation at the site of injection were noted in all groups of PEG-IFN treated monkeys, and in rabbits injected s/c with PEG-IFN in a local irritation study. Histologically, the most consistent finding was evidence of subcutaneous and/or intramuscular hemorrhage at the injection site, without evidence of either acute or chronic inflammation in either rats, rabbits or cynomolgus monkeys treated with single or repeated injections of up to 14,310 μg/m^2/dose PEG-IFN. Sporadic decreases in bone marrow cellularity, lymphoid depletion in the mesenteric nodes and in the spleen, and cortical atrophy in the thymus, as well as evidence of extramedullary hematopoiesis in the liver and spleen were noted in several animals, without an apparent, significant dose-relationship or clinical sequelae. A loss of detectable IFN activity in the serum and development of neutralizing antibody activity was noted at the end of treatment period in repeat-dose studies in cynomolgus monkeys, with no apparent dose-relationship in either incidence or titer of antibody development induced. PEG-IFN exhibited no evidence of mutagenic potential in five tester strains of *Salmonella typhimurium*, and in *E. coli* strain WP2*uvr*A, using the standard Ames microbial mutagenicity plate incorporation tests. No evidence of clastogenic activity of PEG-IFN was detected in *in vitro* assays using human peripheral blood lymphocytes, or in *in vivo* mouse micronucleus assays. PEG alone had no detectable mutagenic, toxicologic, or teratologic activity in *in vitro* Ames and human peripheral blood leukocyte assays, an in *in vivo* toxicology testing in CD-1 (ICR) mice, Sprague-Dawley rats, New Zealand white rabbits, and cynomolgus monkeys at doses of up to 1138 μg/m^2 SCH 215600 s/c, twice weekly for 13 weeks. Treatment of non-pregnant, female cynomolgus monkeys with 52, 262, or 4239 μg/m^2/dose PEG-IFN every other day for one menstrual cycle inhibited ovarian function in 6/7 monkeys at the highest dose level, as evidenced by lengthening of menstrual cycle duration during the treatment period, irregularities in cycle duration following cessation of treatment, and dose-related decreases in serum estradiol, and progesterone levels.

In conclusion, the pharmacologic and toxicologic profiles of PEG-IFN in rats and cynomolgus monkeys are similar to those observed for the unmodified parent compound, INTRON®-A. Toxicities noted in repeat administration of PEG-IFN include transient decreases in appetite, leukocyte, neutrophil, and lymphocyte counts, increase in menstrual cycle duration, and alterations in serum reproductive hormone levels in female monkeys. Other findings included non-reversible decreases in erythrocyte parameters, inflammation and/or irritation at the injection site, and development of serum neutralizing activity after repeat administration. Based on the hematologic toxicities noted, the NOAEL for PEG-IFN in cynomolgus monkeys after repeat, every other day injections for 28 days was 1414 μg/m^2/dose. The NOAEL for effects of PEG-IFN on reproductive hormone status and menstrual cycle duration in cynomolgus monkeys was 262 μg/m^2/dose, or approximately 21-fold greater than the recommended human weekly dose of 1 mg/kg.

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Key Words: chronic hepatitis C infection; interferon; PEG-interferon; toxicity

concurrences:
OTRR/C, CP-T/MGreen

cc:
OTRR/C, CP-T/MGreen
OTRR/C, IID/WSchwieterman